Neonatology
A Practical Approach to Neonatal Diseases

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Editors

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Springer

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ISBN 978-88-470-1404-6 e-ISBN 978-88-470-1405-3

DOI 10.1007/978-88-470-1405-3

Springer Milan Dordrecht Heidelberg London New York

Library of Congress Control Number: 2011940798

6 5 4 3 2 1 2012 2013 2014 2015 2016

Cover design: Ikona S.r.l., Milano, Italy

Typesetting and production: Scienzaperta S.n.c. Publishing Services, Novate Milanese (Milano), Italy
Printing and binding: Printer Trento S.r.l., Trento, Italy
Printed in Italy

Springer-Verlag Italia S.r.l, Via Decembrio 28, I-20137 Milano, Italy
Springer is part of Springer Science+Business Media (www.springer.com)

Preface

All practitioners, doctors and nurses, who care for newborn babies have an enormous responsibility. They are entrusted with the care of approximately 130 million children who are born each year worldwide, about 20 million of whom are of low birth weight. Because they are born light and early, these children are at high risk of dying during the perinatal period, have reduced survival during infancy and are at risk of survival with cognitive impairment. Their health later in life may be adversely affected and even the next generation may suffer. Some babies become sick because they are born small, others because of inherited diseases, which often provide diagnostic and therapeutic challenges. Still others, about one in every thousand, have thrived during the pregnancy but are subjected to severe and potentially damaging hypoxic ischaemia at full-term. We firmly believe that good care can help improve the survival of these small vulnerable patients and this book was produced with the aim of providing healthcare practitioners in neonatology with an up-to-date, readily available and comprehensive source of practical advice for the expert management of their patients and their diverse illnesses.

Neonatology is a science in rapid evolution and all the contributors to this new textbook are experts in their fields, clinically active and committed to developing the best possible care for their patients. This book brings together their expertise in a clear and easy-to-follow manner. Although some may feel that in the age of the internet such a textbook is an anachronism, we feel passionately that those caring for newborn babies will be helped by the accessibility of the information, written by experts, in this single volume. Our intention is that healthcare practitioners with responsibility for babies will be helped by the knowledge of the contributors to this book and their collective experience acquired over many years. The evidence base for practice has been set out where available, but there are gaps in the research-based evidence and sick babies still deserve to be given first-class care. We firmly believe that this textbook will enable the better delivery of such high quality care by making widely available the personal expertise of the contributors, who have been selected because of their international reputations and renown. We hope this textbook finds a place in the library of every neonatal unit and on the bookshelf of every practitioner committed to the care of newborn babies.

The book has been organized in a logical manner. In the first section, relevant epidemiology and fetal medicine are considered. Here consideration is given to epigenetic mechanisms and well as more traditional risk factors. Intrauterine growth restriction is considered from obstetric, neonatal and therapeutic aspects. There are chapters devoted to the difficult ethical problems in this area of care, how to make decisions, and how to measure the quality of the care that our small patients receive. The vital issue of follow up outcomes is considered, as well as the complexities of cerebral plasticity and functional reorganization in children with congenital brain lesions.

The second section deals with therapeutic issues, including the organization of perinatal care, transport services, resuscitation and oxygen toxicity, home care after discharge from hospital and the training of specialist doctors and nurses. Here legal issues are also considered. The third section on nutrition begins with a consideration of the

physiology of the gastrointestinal tract and of the developing endocrine milieu. Nutrition of the baby in hospital is considered in its various aspects, as is post-discharge nutrition, calcium and phosphorous homeostasis and micronutrients and vitamins. Section IV provides a detailed consideration of the problems due to drugs taken during pregnancy and there is an expert review of developmental pharmacology and therapeutics. Issues relating to infants born to mothers with diabetes are also considered here. Section V deals with the respiratory system in all its complexity, ranging from its development and physiology, through a consideration of the control of breathing and the molecular structure of surfactant and rare lung disorders, to practical therapeutic considerations. The following sections deal with the cardiovascular system, hyperbilirubinemia and liver diseases. Section VIII deals with surgery, including the treatment of orofacial malformations and necrotizing enterocolitis, and pre- and post- natal management. Subsequent sections deal with hematology, immunology and inflammation, and the rare but important topic of neonatal malignancies. Sections on infection and endocrine and metabolic disease follow. Finally there are sections on neurology, including brain development, injury, electrophysiology and imaging, and relevant ophthalmology, orthopedics and dermatology.

This book is comprehensive and provides an easily accessible single reference point. It is intended for all involved in the care of newborn babies – pediatricians, neonatal nurses, nurse practitioners, midwives, pediatric surgeons, anesthesiologists, neonatologists and those training in these various areas.

Siena and Liverpool, November 2011

Giuseppe Buonocore
Rodolfo Bracci
Michael Weindling
Editors

Acknowledgements
The Editors are grateful to the Europe Against Infant Brain Injury (EURAIBI - Onlus) Foundation for its most generous support. Particular thanks to Barbara Marzocchi, who gave so much energy contacting contributors and getting back manuscripts, and to Marco Martorelli for his unfailing help in copy editing. Last, but no means least, we are very grateful to our trainees and residents, Sara Bertrando, Sara Cornacchione, Tei Monica, and Gemma Stazzoni, who carefully checked the index.

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1

Epidemiology: Mortality and Morbidity

Marcello Orzalesi and Carlo Corchia

1.1 Introduction

During the past 20 years the most important indicators of maternal and child health have improved considerably in many parts of the world, with the notable exception of countries affected by the HIV/AIDS epidemic. Nevertheless, it is estimated that out of 130 million babies that are born alive every year, almost 8 million die in their first year of life and more than 4 million die in the first 4 weeks after birth; furthermore, every year over 3,3 million fetuses are stillborn and one third of fetuses die during delivery [1].

The rates of neonatal, infant and child mortality as well as those of stillbirths and perinatal mortality vary considerably among different nations, with a persistent large gap between developed and developing countries [1]. The reasons for these variations are many and complex, including socio-economic, educational, organizational, technological, ethnic, historical as well as being dependent on other characteristics of the different countries. This description focuses on the situation in developed countries: Europe, North America, Japan, Australia and New Zealand. Those who are interested in a description of maternal and child health in the rest of the world are referred to the recent report of the World Health Organization (WHO) [1].

1.2 Definitions

When comparing data from different countries, regions or single centers, discrepancies arise because of differences in the definitions of the variables used for comparison. For this reason, the WHO has recommended using definitions from the tenth revision of the international classification of diseases (CD) as adopted by the World Health Assembly [2] (see § 1.7).

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In spite of these WHO recommendations, substantial differences in international criteria for measuring perinatal and neonatal mortality persist [3]. The issue has also been somewhat confused by two other factors: a recent trend towards increasing survival of infants of gestational age (GA) below 26 completed weeks or birth weight below 800 grams [4, 5], and variability in attitudes concerning resuscitation of these infants, which depends on different personal or institutional ethical approaches [6, 7]. These factors have made it difficult to compare stillbirth and neonatal death rates between single centers or geographically defined areas.

1.3 Sources of Data

The data for the evaluation of perinatal and neonatal health are derived from two main sources: center-based, i.e., data from single or multiple centers; and area-based, i.e., data from a geographically defined area, such as a region or a whole country.

The first type of data is more easily obtainable and therefore common in the literature. Such data are useful for monitoring the performance of a single center over time, for comparisons and bench-marking between different centers, for the study of specific risk factors and for the evaluation of new procedures or therapeutic approaches. However, they are subject to selection biases and, since they are usually provided by centers of excellence, the results are generally better than those obtained from area-based studies and do not provide a true picture of the overall situation in a given region or country [8].

The second type of data is less easily obtained, since it requires considerable organizational and financial efforts, and such data are therefore less common in the literature. They are, however, extremely useful since they provide a more complete picture of the situation in a given region or country, including highlighting important socio-economic and organizational aspects that are often not apparent from center-based studies.

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

1.4 Neonatal Mortality Rates

Over the past decades, the neonatal mortality rate (NMR, within 28 days of birth) has decreased steadily in most parts of the world. However, the gap between developed and developing countries persists [9], and in some cases has widened (Table 1.1). Significant differences have also been reported between the most developed countries (Table 1.2) [9] and between different geographical areas of the same country [10, 11]. Once again, the reasons for these differences are many and often not entirely clear: socio-economic, demographic and organizational issues play their parts, as do different attitudes towards the more or less aggressive management of infants of very low GA at the limits of viability. However, these factors do not entirely explain the variability.

The contribution of neonatal mortality to infant mortality rate (IMR, within the first year of life) and to child mortality rate (CMR, within the first 5 years of life) also varies greatly between countries, and neonatal mortality continues to be a major cause of death in infancy and early childhood all over the world (Tables 1.1 and 1.2).

The causes of neonatal deaths vary between countries (Table 1.3) [9]. In developed countries four main causes account for more than 75% of all neonatal deaths: disorders related to prematurity or low birth weight (LBW), congenital anomalies, neonatal infections, birth asphyxia and birth trauma [9]. The proportion to which each one of these causes contributes to neonatal mortality varies from country to country but prematurity and LBW are always predominant.

1.5 Prematurity and Low Birth Weight

Prematurity (GA <37 weeks) and LBW (<2500 g) are the leading causes of neonatal and infant death among babies born in developed countries [9, 12]. Neonatal and infant mortality for moderately LBW infants (BW 1500–2499 g) is five times higher than for infants of normal birthweight: the mortality of LBW infants is almost completely concentrated in infants of very low birth weight (VLBWI <1500 g) who are 100 times more likely to die than their normal birth weight counterpart [13].

The contribution of prematurity and LBW to the total neonatal mortality appears to be greater when the NMR is lower [9], and this is particularly true for VLBW infants who contribute to more than half the neonatal and infant deaths in developed countries with a low NMR [13]. For these reasons, neonatologists have concentrated their attention on VLBW infants, and particularly on those of extremely low birth weight (ELBWI, <1000 g). The prevalence of VLBW infants is around 1–2% of all live births but varies considerably between countries, and this can significantly affect the neonatal mortality rates [14]. The neonatal mortality rate also varies between centers and geographically defined areas; part of this variation is due to differences in the management of these infants in NICU, but a great part can also be attributed to selection biases [15] and differences in reporting [14].

Neonatal mortality increases progressively with a lowering of GA and BW. The inverse proportion between neonatal mortality and GA is well depicted in a recent study from England and Wales [16]. In general, for any given GA, the lower the BW, the higher the mortality; and for any given BW, the lower the GA, the higher the mortality [12]. The prognosis is therefore usually worst in small for gestational age infants (SGA, BW <10th percentile for GA).

Many other factors affect the survival of preterm and LBW infants. These include gender, ethnicity, multiple pregnancy, maternal health and complications of pregnancy, mode and place of delivery, prenatal administration of corticosteroids, condition at birth and clinical problems after birth

Table 1.1 Number of births/year (2007), neonatal mortality rates (NMR, 2004), infant mortality rates (IMR, 2007) and child mortality rates under 5 years (CMR, 2007) in different parts of the world (decimals are omitted and numbers are rounded to the nearest unit)

Geographic Area	No. of births/year (×1000)	NMR ‰ (2004)	IMR ‰ (2007)	CMR ‰ (2007)
Sub-Saharan Africa	30,323	41	89	148
(East + South Africa)	14,268	36	80	123
(West + Central Africa)	16,056	45	97	169
Middle East + North Africa	9,726	25	36	46
South Asia	37,986	41	59	78
East Asia + Pacific	29,773	18	22	27
Latin America + Caribbean	11,381	13	22	26
CEE/CIS	5,560	16	22	25
Industrialized Countries	11,021	3	5	6
Developing Countries	122,266	31	51	74
Least Developed Countries	29,076	40	84	130
Whole World	135,770	28	47	68

CEE/CIS Central and Eastern Europe and the Commonwealth of Independent States.
Derived from [9].

Table 1.2 Number of births/year (2007), neonatal mortality rates (NMR, 2004), infant mortality rates (IMR, 2007) and child mortality rates under 15 years (CMR, 2007) in 43 countries with more than 50,000 births/year and with an NMR ≤ 10/1000 live births (decimals are omitted and numbers rounded to the nearest unit)

Country	No. of births/year (×1000)	NMR ‰ (2004)	IMR ‰ (2007)	CMR ‰ (2007)
Albania	52	9	13	15
Australia	256	3	5	6
Austria	77	3	4	4
Belarus	91	3	12	13
Belgium	109	2	4	5
Bulgaria	68	7	10	12
Canada	340	3	5	6
Cuba	118	4	5	7
Czech Republic	93	2	3	4
Denmark	62	3	4	4
Finland	58	2	3	4
France	758	2	4	4
Germany	678	3	4	4
Greece	103	3	4	4
Hungary	93	5	6	7
Ireland	67	4	4	4
Israel	137	3	4	5
Italy	539	3	3	4
Jamaica	55	10	28	33
Japan	1,070	1	3	4
Kuwait	51	7	9	11
Malaysia	555	5	10	11
Netherlands	184	3	4	5
New Zealand	57	3	5	6
Norway	56	2	3	4
Oman	58	5	11	12
Poland	360	5	6	7
Portugal	112	3	3	4
Republic of Korea	448	4	4	5
Romania	211	10	13	15
Russian Federation	1,515	7	13	15
Serbia	127	9	7	8
Slovakia	53	4	7	8
Spain	476	2	4	4
Sri Lanka	292	8	17	21
Sweden	102	2	3	3
Switzerland	69	3	4	5
Thailand	932	9	6	7
Ukraine	419	7	20	24
United Arab Emirates	71	4	7	8
United Kingdom	722	3	5	6
United States	4,281	4	7	8
Uruguay	51	7	12	14

Derived from [9].

Table 1.3 Major causes of neonatal deaths (% of total neonatal deaths) in different WHO regions

WHO Region	A	B	C	D	E	F	World
Cause of death (%)							
Prematurity and LBW	26	33	25	34	35	37	31
Neonatal Infections	27	23	29	18	24	19	26
Birth asphyxia & trauma	24	14	21	19	23	25	23
Congenital anomalies	6	14	9	15	5	7	7
Diarrheal diseases	3	–	4	1	2	1	3
Neonatal tetanus	5	–	4	–	3	2	3
Other non infectious	9	15	8	13	8	9	7

A African regions, *B* Americas, *C* Eastern Mediterranean regions, *D* European regions, *E* South East Asia, *F* Western Pacific.
Derived from [9]. For the complete list of the nations pertaining to each WHO Region see [1].

[12]. The frequency and severity of clinical problems tend to be related more to GA than BW [12].

The mortality rate of LBW infants has declined progressively during the past decades. Improvements in the care of preterm fetuses and newborns have contributed substantially to this decline [12]. Regionalization of perinatal care, with increased numbers of infants delivered in third level hospitals with intensive care units [12, 17], has also contributed significantly. Efforts to prevent prematurity and LBW have not been equally successful. This is because the causes and risk factors associated with preterm and LBW births are numerous and by and large unknown or not completely understood [12, 17, 18]. They are usually grouped into three major categories: spontaneous preterm labor and rupture of membranes, maternal and fetal complications, and multiple pregnancy and assisted reproduction [18]. In many developed countries, there has been a slight increase in preterm births mainly due to increasing rates of multiple births, greater use of assisted reproduction techniques, and more obstetric interventions [12, 18, 19].

Premature and LBW infants contribute to more than half of all neurodevelopmental, cognitive, sensory and other disabilities in infancy, childhood and adolescence [12]. The incidence and severity of such long-term sequelae, as reported by single or multicenter studies, varies according to the case mix, the obstetric and neonatal management, the age and methods of evaluation and other variables [18–20]. In general, the incidence and severity are inversely correlated with GA and BW and are particularly high in infants of extremely low GA (<26 weeks) (see Chapter 129).

There has been some concern that the increased survival of these high risk and delicate infants would result in an increased number of infants with long-term severe disabilities. This appeared indeed to be the trend until the early 1990s; however, the trend seems to have reversed and recent data indicate that improved survival may be associated with a concomitant decrease in cerebral palsy and other disabling conditions [21–23].

Recent technological advances in neonatal intensive care have pushed back the lower limits of gestation at which survival is possible and many centers have reported significant survival in infants of 23 and even 22 weeks' gestation. However, as shown by the few area-based studies where follow-up has been carried out to around school age, the short and long-term prognosis of these infants at the limits of viability (GA <25 weeks) remains very poor [24–26].

1.6 Conclusions

Perinatal, neonatal and infant mortality rates have decreased in the past decade in many parts of the world but remain high in most developing countries. Neonatal mortality contributes significantly to infant and child mortality and morbidity, particularly in developed countries.

In developing countries some of the causes of intrapartum and neonatal deaths, such as birth asphyxia and birth trauma, neonatal infections, tetanus and diarrheal diseases, are largely preventable by adequate social, organizational, hygienic, obstetric and neonatal interventions.

In developed countries, the challenge concerns mainly premature infants of very low gestation. Their numbers seem to have increased because of more widespread use of assisted reproduction techniques and because of more aggressive obstetric and neonatologic approaches.

It is unlikely that the mortality rates of these infants can be further significantly decreased by new therapies or technological advances. However, it should be possible to decrease the incidence and severity of long-term sequelae.

Hopes for a significant improvement in neonatal mortality depend mainly on the prevention of prematurity and low birth weight, although this seems to be a goal difficult to achieve.

Careful standardized collection and reporting of area based data relating to maternal, perinatal, neonatal and infant mortality and morbidity, such as those published in the European Perinatal Health Report [27], is highly recommended and may help identify the areas and types of interventions that might be reasonably expected to improve maternal and child health.

1.7 Appendix

Extracted from the International statistical classification of diseases and related health problems, 10th revision (ICD-10) [2]

Definitions

The following definitions have been adopted by the World Health Assembly in relation both to statistics amenable to international comparison and to reporting requirements for the data from which they are derived.

- *Live birth*. Live birth is the complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of the pregnancy, which, after such separation, breathes or shows any other evidence of life, such as beating of the heart, pulsation of the umbilical cord, or definite movement of voluntary muscles, whether or not the umbilical cord has been cut or the placenta is attached; each product of such a birth is considered liveborn.
- *Stillbirth or fetal death (deadborn fetus)*. Fetal death is death prior to the complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of pregnancy; the death is indicated by the fact that after such separation the fetus does not breathe or show any other evidence of life, such as

beating of the heart, pulsation of the umbilical cord, or definite movement of voluntary muscles.

- *Birth weight*. The first weight of the fetus or newborn obtained after birth. For live births, birth weight should preferably be measured within the first hour of life before significant postnatal weight loss has occurred. While statistical tabulations include 500 g groupings for birth weight, weights should not be recorded in those groupings. The actual weight should be recorded to the degree of accuracy to which it is measured.
- *Low birth weight*. Less than 2500 g (up to and including 2499 g).
- *Very low birth weight*. Less than 1500 g (up to and including 1499 g).
- *Extremely low birth weight*. Less than 1000 g (up to and including 999 g).
- *Gestational age*. The duration of gestation is measured from the first day of the last normal menstrual period. Gestational age is expressed in completed days or completed weeks (e.g., events occurring 280–286 completed days after the onset of the last normal menstrual period are considered to have occurred at 40 weeks of gestation). Gestational age is frequently a source of confusion, when calculations are based on menstrual dates. For the purposes of calculation of gestational age from the date of the first day of the last normal menstrual period and the date of delivery, the first day is day zero and not day one; therefore days 0–6 correspond to "completed week zero"; days 7–13 to "completed week one"; and the 40th week of actual gestation is synonymous with "completed week 39". Where the date of the last normal menstrual period is not available, gestational age should be based on the best clinical estimate. In order to avoid misunderstanding, tabulations should indicate both weeks and days.
- *Preterm*. Less than 37 completed weeks (less than 259 days) of gestation.
- *Term*. From 37 completed weeks to less than 42 completed weeks (259–293 days) of gestation.
- *Post-term*. 42 completed weeks or more (294 days or more) of gestation.
- *Perinatal period*. The perinatal period commences at 22 completed weeks (154 days) of gestation (the time when birth weight is normally 500 g), and ends seven completed days after birth.
- *Neonatal period*. The neonatal period commences at birth and ends 28 completed days after birth. Neonatal deaths (deaths among live births during the first 28 completed days of life) may be subdivided into early neonatal deaths, occurring during the first seven days of life, and late neonatal deaths, occurring after the seventh day but before 28 completed days of life.

Neonatal and Perinatal Mortality Rates

Published rates should always specify the denominator, i.e., live births or total births (live births plus fetal deaths). Countries are encouraged to provide the rates listed below, or as many of them as their data collection systems permit.

- *Fetal death rate*. Fetal deaths/1000 total births.
- *Early neonatal mortality rate*. Early neonatal deaths/1000 live births.
- *Perinatal mortality rate*. Fetal deaths and early neonatal deaths/1000 total births. The perinatal mortality rate is the number of deaths of fetuses weighing at least 500 g (or, when birth weight is unavailable, after 22 completed weeks of gestation or with a crown-heel length of 25 cm or more), plus the number of early neonatal deaths, per 1000 total births. Because of the different denominators in each component, this is not necessarily equal to the sum of the fetal death rate and the early neonatal mortality rate.
- *Neonatal mortality rate*. Neonatal deaths/1000 live births. It can be subdivided into early mortality rate (first week of life) or late mortality rate (8–28 days of life).
- *Infant mortality rate*. Deaths under one year of age/1000 live births.

Presentation of Causes of Perinatal Mortality

For statistics of perinatal mortality derived from the certificate recommended for this purpose, full-scale multiple-cause analysis of all conditions reported are of greatest benefit. Where such analysis is impracticable, analysis of the main disease or condition in the fetus or infant and of the main maternal condition affecting the fetus or infant, with cross-tabulation of groups of these two conditions should be regarded as the minimum. Where it is necessary to select only one condition (for example, when early neonatal deaths must be incorporated into single-cause tables of deaths at all ages), the main disease or condition in the fetus or infant should be selected.

Reporting Criteria

The legal requirements for the registration of fetal deaths and live births vary from country to country and even within countries. If possible, all fetuses and infants weighing at least 500 g at birth, whether alive or dead, should be included in the statistics. When information on birth weight is unavailable, the corresponding criteria for gestational age (22 completed weeks) or body length (25 cm crown-heel) should be used. The criteria for deciding whether an event has taken place within the perinatal period should be applied in the order: (1) birth weight, (2) gestational age, (3) crown–heel length. The inclusion of fetuses and infants weighing between 500 g and 999 g in national statistics is recommended both

because of its inherent value and because it improves the coverage of reporting at 1000 g and over.

Statistics for International Comparison

In statistics for international comparison, inclusion of the extremely low birth weight group disrupts the validity of comparisons and is not recommended. Countries should arrange registration and reporting procedures so that the events and the criteria for their inclusion in the statistics can be easily identified. Less mature fetuses and infants not corresponding to these criteria (i.e., weighing less than 1000 g) should be excluded from perinatal statistics unless there are legal or other valid reasons to the contrary, in which case their inclusion must be explicitly stated. Where birth weight, gestational age and crown-heel length are not known, the event should be included in, rather than excluded from, mortality statistics of the perinatal period. Countries should also present statistics in which both the numerator and the denominator of all rates are restricted to fetuses and infants weighing 1000 g or more (weight-specific rates). Where information on birth weight is not available, the corresponding gestational age (28 completed weeks) or body length (35 cm crown–heel) should be used. In reporting fetal, perinatal, neonatal and infant mortality statistics the number of deaths due to malformations should whenever possible be identified for live births and fetal deaths and in relation to birth weights of 500–999 g and 1000 g or more. Neonatal deaths due to malformations should be subdivided into early and late neonatal deaths. This information enables perinatal and neonatal mortality statistics to be reported with or without the deaths from malformations.

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2

The Development from Fetus to Newborn

Domenico Arduini and Marianne Vendola

2.1 The Fetus at Term

The stages of development of the human, from embryo to fetus and finally to newborn baby, are shown in Table 2.1.

Table 2.1 The stages of development of the human fetus, from embryo to newborn

Fetus at term
1. Development phases of the fetus
2. Fetal constitutional characteristics
3. Diagnosis of fetal well-being
4. Fetal injuries
5. Fetal response to injuries
6. Postnatal development

2.2 Developmental Phases of the Fetus

The embryonic phase is a critical stage of development, when systems undergo important basic development.

The third week after conception marks the beginning of the embryonic period. It ends at the end of the tenth week, when the embryo comprises three layers from which all organs will develop.

The second fetal phase begins after the tenth week and continues until the end of pregnancy. During this phase, organs (liver, kidneys) begin to function.

From the 16th to 20th weeks, the fetus undergoes a rapid growth spurt. Fat develops under a thin skin. Cardiac output increases. Meconium accumulates in the bowel. The fetus hiccups and spends time awake and asleep.

Fetal development slows down between the 21st and 24th weeks. By the 24th week, the fetus weighs approximately 1,3 pounds (0,6 kg).

Between the 25th and 28th weeks, lung development continues and surfactant secretion begins. By the 28th week, 90% of fetuses will survive *ex utero* with appropriate support.

From the 29th to the 40th weeks, the amount of body fat rapidly increases. Thalamic brain connections, which mediate sensory input, form. Bones are fully developed. Most of the major systems and organs are complete. The immune system develops.

By weeks 35–40, the fetus is sufficiently developed for life outside the uterus without any more support than that which would be required by any newborn baby delivered at term. At 37 weeks, the fetus will continue to add approximately one ounce (28 g) per day to its body weight and it will be 48–53 cm (19–21 inches) in length at birth.

2.3 Fetal Constitutional Characteristics

2.3.1 The Central Nervous System (CNS)

The CNS is formed by four subdivisions of the neural tube that develop into distinct regions of the central nervous system.

The neural tube is initially open both cranially and caudally. These openings close during the fourth week. Failure of closure of these neuropores can result in neural tube defects such as anencephaly or spina bifida.

The dorsal part of the neural tube comprises the alar plate, which is primarily associated with sensation. The ventral part of the neural tube comprises the basal plate, which is primarily associated with motor control.

The spinal cord is a long, thin, tubular bundle of nervous tissue and support cells that extend from the brain. The brain and spinal cord together make up the central nervous system. The spinal cord functions primarily for the transmission of neural signals between the brain and the rest of the body, but also contains neural circuits that independently control numerous reflexes and central pattern generators.

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

2.3.2 The Fetal Circulation

The essential difference between the circulatory system of a fetus and that of the baby after birth is that the lungs are not in use: the fetus obtains oxygen and nutrients from the mother through the placenta and the umbilical cord. Blood from the placenta is carried to the fetus by the umbilical vein. A large proportion enters the fetal ductus venosus and passes to the inferior vena cava, while the remainder enters the liver from vessels on its inferior border. The branch of the umbilical vein that supplies the right lobe of the liver joins the portal vein and blood then passes to the right atrium. In the fetus, there is an opening between the right and left atria (the foramen ovale), and most of the blood flows from the right into the left atrium, thus bypassing pulmonary circulation. The majority of blood flow is then into the left ventricle from where it is pumped through the aorta to supply the various organs. Blood then flows from the aorta through the internal iliac arteries to the umbilical arteries, and re-enters the placenta, where carbon dioxide and other waste products from the fetus are taken up and enter the maternal circulation. A small proportion (about 4%) of the blood from the right atrium does not enter the left atrium, but enters the right ventricle and is pumped into the pulmonary artery. In the fetus, a connection between the pulmonary artery and the aorta, called the ductus arteriosus, directs most of the blood away from the lungs (which are not being used for respiration at this point as the fetus is suspended in amniotic fluid).

An important concept of the fetal circulation is that fetal hemoglobin has a higher affinity for oxygen than adult hemoglobin, which facilitates diffusion of oxygen from the maternal circulation to the fetus. The circulatory system of the mother is not directly connected to that of the fetus, so gas exchange takes place at the placenta. Oxygen diffuses from the placenta to the chorionic villus, an alveolus-like structure, from which it is then carried to the umbilical vein. Fetal hemoglobin enhances the fetal ability to draw oxygen from the placenta because the oxygen dissociation curve is shifted to the left, which has the effect of oxygen being taken up at a lower concentration than by adult hemoglobin. This enables fetal hemoglobin to take up oxygen from adult hemoglobin in the placenta, which has a lower partial pressure of oxygen than at the lungs after birth.

A developing fetus is highly susceptible to anomalies of growth and metabolism, increasing the risk of birth defects.

2.3.3 Fetal Metabolism

A continuous placental supply of glucose provides the substrate for energy metabolism to the fetus, and this converts after birth to intermittent feeding. While the fetus is dependent on maternal glucose as the main source of energy, it can also use lactate, free-fatty acids, and ketone bodies under some conditions (e.g. starvation or hypoxia). Fetal glucose utilization is augmented by insulin produced by the developing fetal pancreas in increasing amounts as gestation proceeds; this enhances glucose utilization in insulin-sensitive tissues (skeletal muscle, liver, heart, adipose tissue), which increase in mass and thus glucose requirement during late gestation. Glucose-stimulated insulin secretion increases with gestation. Glycogen stores are maximal at term, but even the term fetus only has sufficient glycogen available to meet energy needs for 8–10 hours, and this store can be depleted even more quickly if demand is high. At 27 weeks' gestation, only 1% of a fetus's body weight is fat; this increases to 16% at 40 weeks. Inadequate glucose substrate can lead to hypoglycemia and fetal growth restriction. In cases of intrauterine growth restriction, fetal weight-specific tissue glucose uptake rates and glucose transporters are maintained or increased, while synthesis of amino acids into protein and corresponding insulin-like growth factor (IGF) signal transduction proteins are decreased. These observations demonstrate the mixed phenotype of the intrauterine growth restriction (IUGR) fetus that has an enhanced capacity for glucose utilization, but a diminished capacity for protein synthesis and growth. Excess substrate can also lead to problems, as with infants of diabetic mothers (IDM). Thus, the normal fetus has a considerable capacity to adapt to changes in glucose supply [1].

2.3.4 Regulation of Fetal Growth

Fetal growth depends on many different aspects, mostly influenced by maternal and uteroplacental factors.

2.3.4.1 Role of the Mother in Fetal Growth Regulation

Fetal growth and development are influenced by genetic as well as environmental factors. Maternal genes have an important specific influence on fetal growth; for example, maternal height is a major determinant of fetal size, representing uterine capacity and the potential for growth. Although the birth weights of siblings are similar and correlate, environmental influences are also important in determining growth. Maternal constraint refers to the limited capacity of the uterus to support fetal growth and is important in limiting fetal overgrowth and subsequent dystocia, to ensure the mother's capacity for future successful pregnancies [2].

Maternal Nutrient Intake

The mother is the supplier of oxygen and essential nutrients to the fetus via the placenta. Maternal diet, caloric intake, and metabolic function have an important role in supplying nutrients to the fetus. Increased caloric intake is necessary during the second and third trimesters to allow for fetal and

placental growth [3]. A Cochrane systematic review of six randomized controlled trials found that balanced protein-energy supplementation reduced the risk of small for gestational age (SGA) neonates by approximately 30% [4]. Glucose is an important nutrient in the control of fetal growth. Studies of diabetic women have shown that low blood glucose levels during pregnancy as a result of excessively tight glycemic control lead to a greater incidence of SGA neonates, whereas high blood glucose levels increase the likelihood of macrosomia [5].

Maternal Uterine Artery Blood Flow

Increased uterine blood flow is essential to meet the metabolic demands of the growing uterus as well those of the placenta and fetus [6]. Uterine artery blood flow increases by more than three-fold during pregnancy, partly influenced by an increased artery diameter and reduced resistance to flow. In addition to increased uterine blood flow during normal pregnancy, new blood vessels develop in the uterus, promoted by the placental hormones human chorionic gonadotropin (hCG) [7] and IGF-II [8]. Using Doppler assessment of uterine arterial flow at 23 weeks' gestation, Albaiges et al [9] found that that increased uterine artery blood flow resistance was associated with an increased risk of an SGA baby.

Maternal Smoking and Drug Use

Maternal cigarette smoking is associated with reduced birth weight. Early reports suggested a doubling of the rate of low birth weight in babies of smokers compared with those of non-smokers and a dose-dependent effect with increasing number of cigarettes smoked. More recent studies demonstrated a 3.5-fold increased risk of SGA infants in women who smoked during pregnancy [10], with a greater effect on low birth weight with increasing maternal age [11]. Growth restriction is usually symmetrical with reduced weight, head circumference, and abdominal circumference. The use of drugs, such as cocaine and marijuana, also has significant negative effects on fetal growth. Cocaine use contributes to an increased rate of low birth weight and a reduction in mean birth weight of at least 100 g.

Maternal Hypoxia

Maternal hypoxia influences fetal growth. Its effect is independent of socioeconomic status, prematurity, maternal smoking, pregnancy-induced hypertension, and parity. The combination of hypoxia and pregnancy appears to be important in altering maternal physiology, including changes in immune pathways [12]. Maternal hypoxia affects placental and uterine blood flow, which contribute to reduced nutrient transport to the fetus [13].

Maternal Inflammatory Diseases

The presence of maternal inflammatory disease may contribute to reduced fetal growth. Several inflammatory diseases are associated with reduced fetal growth, including rheumatoid arthritis [14], inflammatory bowel disease, systemic lupus erythematosus, and periodontal disease [15]. Women with active inflammatory arthritis during pregnancy have smaller babies compared with healthy women or women whose disease is in remission [16], suggesting that active inflammation during pregnancy may contribute to reduced fetal growth. Maternal health influences the maternal state during pregnancy with implications for fetal growth. In addition to inflammatory diseases, many other maternal factors, including preeclampsia [17], anemia [18], infections and alcohol consumption, influence fetal growth via changes in placental function.

2.3.4.2 Role of the Placenta in Fetal Growth Regulation

The placenta receives and transmits endocrine signals between the mother and fetus and is the site of nutrient and waste exchange. Adequate placental growth is essential for adequate fetal growth. Several aspects of placental function are critical for human fetal growth and development, including adequate trophoblast invasion, an increase in uteroplacental blood flow during gestation, transport of nutrients such as glucose and amino acids from mother to fetus, and the production and transfer of growth-regulating hormones. Increased blood flow during pregnancy increases the flow of nutrients from mother to fetus, and uteroplacental blood flow has been shown to be reduced by up to 50% in women with preeclampsia [19]. Doppler velocimetry is used to detect increased vascular resistance in the uterine arteries, which occurs as a result of abnormal trophoblast invasion of the spiral arteries. In addition, examination of the fetal circulation, particularly umbilical artery waveforms, may reflect placental insufficiency [20]. Umbilical vein blood flow, measured by Doppler ultrasound, is decreased in IUGR fetuses, representing reduced perfusion of the fetal tissues.

Placental Hormone Production

During human pregnancy, the placenta is an important endocrine organ. It produces hormones, including estrogens and progesterone, hCG, human growth hormone (GH) variant, and human placental lactogen. Some of these play a role in the regulation of fetal growth. Fetal insulin promotes growth of the fetus, acting as a signal of nutrient availability [20]. Insulin deficiency results in reduced fetal growth, as the fetal tissues decrease their uptake and utilization of nutrients. There is also a relationship between increased insulin production and increased fetal growth. It has been proposed that the fetus increases its own production of insulin in response to maternal hyperglycemia, and that this increase in fetal insulin

is responsible for the increased growth and macrosomia observed in diabetic pregnancies.

2.4 Diagnosis of Fetal Well-being

During pregnancy, women are generally offered non-invasive screening tests, such as blood tests, ultrasound and cardiotocography (CTG) (to detect the fetal heartbeat and uterine contractions, usually monitored during the third trimester), to evaluate the baby's health. Alternatively, more invasive tests, such as chorionic villous sampling or amniocentesis, may be performed.

Obstetric ultrasound is usually used to:

- diagnose pregnancy;
- assess possible risks to the mother (miscarriage or molar pregnancy);
- check for fetal malformation;
- determine intrauterine growth restriction;
- note the development of fetal body parts;
- check the amniotic fluid and the umbilical cord.

Generally an ultrasound examination is ordered whenever an abnormality is suspected or following a schedule similar to that outlined below:

- *7 weeks* Confirm pregnancy, determine expected date of delivery;
- *11–13 weeks* Evaluate the possibility of Down syndrome;
- *20–22 weeks* Perform a scan to assess anatomic integrity;
- *32 weeks* To evaluate fetal growth, verify the position of the placenta and perform the Doppler study to establish fetal well-being.

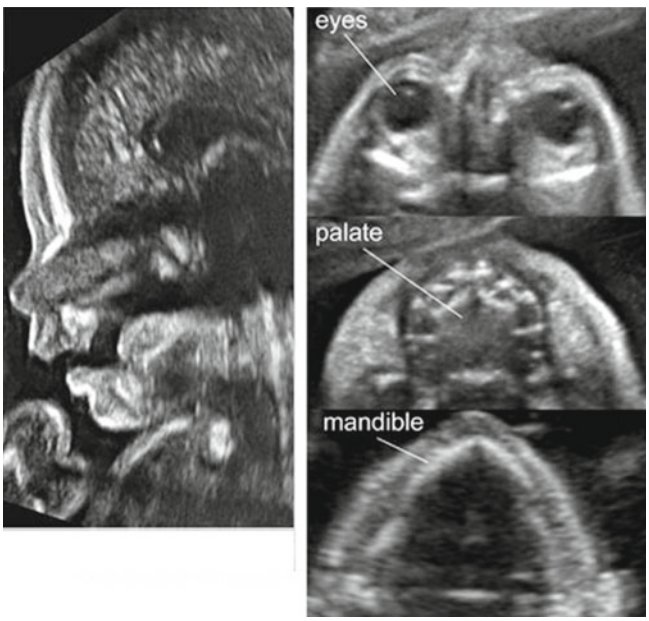

Fig. 2.1 Reconstructed 3-D imaging of the eyes, palate and mandible from the fetal profile

Three- dimensional (3-D) and four- dimensional (4-D) ultrasound techniques are used to provide additional imaging of fetal structures. Today 3-D ultrasound is most commonly performed for the visualization of the baby's face. However, it has the potential to become part of routine care and many hospitals use the 3-D ultrasound to detect fetal anomalies, especially of the heart and of the CNS (Fig. 2.1).

2.5 Fetal Injuries

It is important during fetal development to maintain good fetal oxygen delivery to avoid irreversible fetal compromise. Fetal hypoxia from any cause leads to conversion from aerobic to anaerobic metabolism, which produces less energy and more acid. If the oxygen supply is not restored, the fetus dies. Hypoxia may be:

1. *Hypoxemic hypoxia*: reduced placental perfusion with maternal blood and consequent decrease in fetal arterial blood oxygen content due to low pO_2.
2. *Anemic hypoxia*: reduced arterial blood oxygen content due to low fetal hemoglobin concentration.
3. *Ischemic hypoxia*: reduced blood flow to the fetal tissues.

Making this diagnose can be difficult, and some episodes of hypoxia before and during birth may pass unnoticed at the time, but may affect the central nervous system and not become evident until much later in life.

2.5.1 Causes of Hypoxia

Two major categories of neurological injury can be observed in the full-term infant: (1) hypoxic-ischemic encephalopathy (HIE) and (2) intracranial hemorrhage (ICH). Brain hypoxia and ischemia due to systemic hypoxemia, reduced cerebral blood flow (CBF), or both are the primary pathophysiological processes that lead to an hypoxic-ischemic encephalopathy.

The first compensatory adjustment to an hypoxic-ischemic (asphyxic) event is an increase in CBF due to hypoxia and hypercapnia. This is associated with a redistribution of cardiac output so that the brain receives an increased proportion of the cardiac output. This is followed by a slight increase in systemic blood pressure (BP) due to increased release of epinephrine. In the fetus suffering from acute asphyxia (hypoxic ischemia), if early compensatory adjustments fail, cerebral blood flow (CBF) may become pressure-passive and brain perfusion becomes dependent on systemic BP. As BP falls, CBF falls below critical levels, and a diminished blood supply in the brain leads to insufficient oxygen to meet its needs and intracellular energy failure.

Neuronal injury in hypoxic ischemia is an evolving process. During the early phases of brain injury, brain temperature drops, and there is local release of neurotransmitters, such as γ-aminobutyric acid transaminase (GABA). The magnitude

of the final neuronal damage depends on both the severity of the initial insult and damage due to energy failure, injury during reperfusion, and apoptosis. The extent, nature, severity, and duration of the primary injury are all important in determining the magnitude of the residual neurological damage.

Intracranial hemorrhage in the full-term infant can be intraventricular, subarachnoid, subdural, or intracerebellar. There is often ventilatory disturbance and hypoxia because of varying neurological depression. Intraventricular hemorrhage (IVH), which is unusual in term infants, may be associated with evidence of intrapartum asphyxia, but may also be clinically silent and underdiagnosed, causing later deficits or hydrocephalus [21].

Approximately 20% of neonatal HIE is primarily related to antepartum events that lead to hypoxic ischemia. Maternal conditions such as hypotension, placental vasculopathy, and insulin-dependent diabetes mellitus may predispose the fetus to intrapartum hypoxic ischemia because there is little reserve to compensate for the stresses of labour [22]. Intrapartum events such as prolapsed cord, abruptio placentae, and traumatic delivery have been linked to 35% of cases of HIE. Because of the limitations in determining the actual timing of the insult, it may be difficult to identify the antepartum contribution separately from the intrapartum. Other events besides intrapartum hypoxia may be responsible for HIE or CP, as less than 25% of these infants have symptoms of hypoxic ischemia at birth [23].

The true incidence of intracranial hemorrhage (ICH) in utero has not been determined. Significant subarachnoid hemorrhage can occur with intrapartum hypoxia, or may result from trauma at delivery. It can be isolated or associated with subdural bleeding and cerebral contusion. The presentation is variable but generally includes CNS depression, irritability, and seizures. When subarachnoid hemorrhage is associated with other signs of physical injury and is caused by a difficult delivery, outcome is frequently poor.

In fetal hypoxemia, there is a redistribution of blood flow. This results in an increased blood supply to the brain, myocardium, adrenal glands and reduced perfusion of the kidneys, gastrointestinal tract and the lower extremities. There is preferential delivery of nutrients and oxygen to vital organs, compensating for a diminished placental supply [24]. This compensation is manifest as cerebral vasodilatation and there is an increase in the pulsatility index (PI) in cerebral vessels. The PI index is an arterial blood-flow velocity waveform index designed to quantify the pulsatility or oscillations of the waveform. It is calculated by the formula PI = (Vmax – Vmin)/Vmax mean, where Vmax is the peak systolic velocity, Vmin is the minimum forward diastolic velocity in unidirectional flow, or the maximum negative velocity in diastolic flow reversal, and Vmax mean is the maximum velocity averaged over one cardiac cycle (Figs. 2.2 and 2.3). Cerebral vasodilatation produces a decrease in left ventricular afterload, while increased placental and systemic resistance result in an increased right ventricular afterload. In severe

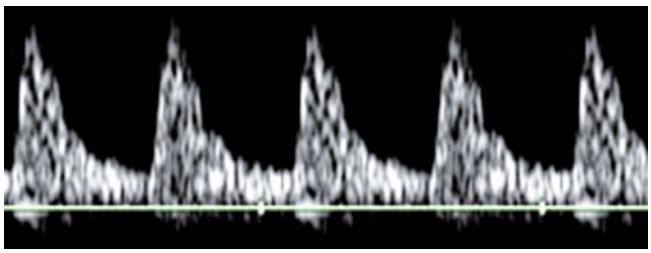

Fig. 2.2 Flow velocity waveforms from the middle cerebral artery in a normal fetus

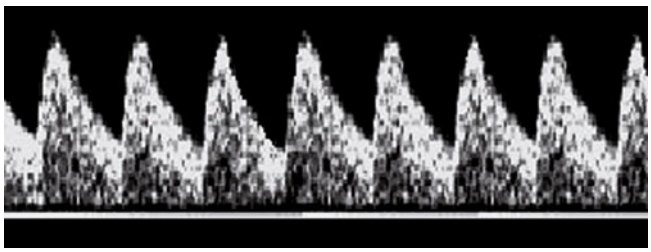

Fig. 2.3 Flow velocity waveforms from the middle cerebral artery in a growth-restricted fetus

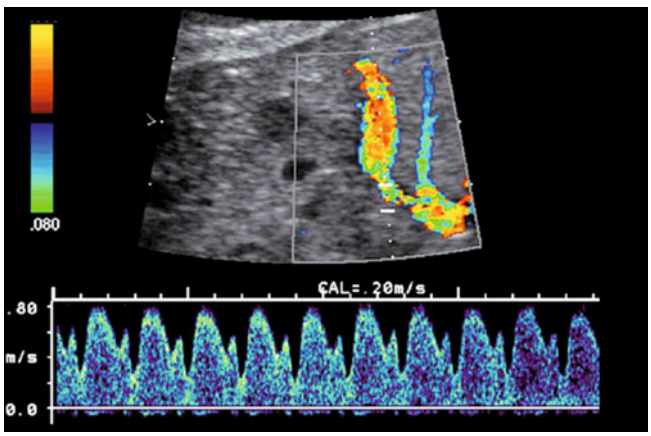

Fig. 2.4 Color Doppler examination of the ductus venosus with normal flow velocity waveforms

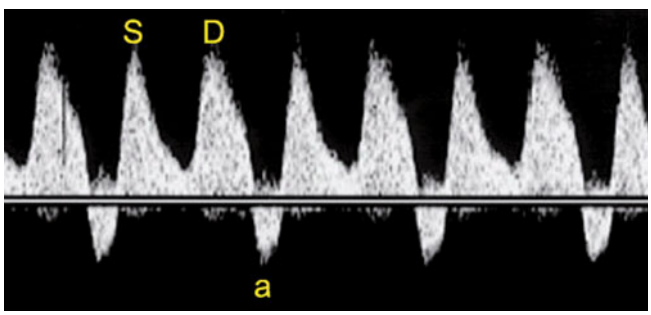

Fig. 2.5 Abnormal DV wave form with reversal of flow during atrial contraction and markedly increased pulsatility systole (*S*), diastole (*D*), atrial contraction (*a*)

hypoxemia, there is also redistribution of umbilical venous blood towards the ductus venosus (Figs. 2.4 and 2.5). Consequently, blood flow in the umbilical vein, which contributes to the fetal cardiac output, is increased. In contrast, a reduced afterload is associated with an increase in peak diastolic forward flow, indicating that fetal systemic vascular resistance has a major influence on venous return and filling patterns of the right heart. Increased placental resistance and peripheral vasoconstriction cause an increase in right ventricular afterload, and thus ventricular end-diastolic pressure increases. This may result in highly pulsatile venous blood flow waveforms and umbilical venous pulsations due to the transmission of atrial pressure waves through the ductus venosus.

2.6 Fetal Response to Injury

During normal development, cardiovascular and circulatory functions progress from fetal life, which is characterized by low PaO_2 (20–24 mmHg; 2.66–3.19 kPa) through transition at birth, to normoxemia after birth (PaO_2 70–80 mmHg; 9.31–10.64 kPa); the fetus and newborn are clearly able to thrive despite their "hypoxic" environment. Adaptive responses by the cardiovascular, metabolic and endocrine systems, allow fairly severe intrauterine hypoxic stress to be tolerated, with the fetus having relatively normal growth and development. However, severe acute or chronic intrauterine hypoxic stress in utero can cause compromised circulation, organ dysfunction, and threaten survival or intact survival. At the time of transition to extrauterine life, signs of a depressed circulatory system because of intrauterine hypoxia may become apparent because of the increased metabolic demands at birth, and loss of placental gas exchange [25]. Acute hypoxemia produces various circulatory adaptations in the fetus that enhance fetal survival, including the development of tachycardia, hypertension, redistribution of blood flow toward the brain, myocardium and adrenals, and depression of fetal breathing and skeletal muscle activity. The fetal heart also has a greater capacity for anaerobic metabolism than the adult heart [26].

The neonatal brain is more resistant to acute hypoxia than the brain of an older child or adult. Nevertheless, hypoxia affecting the fetus or newborn is a major cause of mortality and chronic neurologic disability. The outcome for infants sustaining cerebral hypoxia and ischemia is influenced by many factors, including the duration and severity of the event, and associated infectious, traumatic, or metabolic (especially hypoglycaemic) derangements [27]. Repetitive episodes of severe hypoxia may cause global neuronal, cortical, midbrain, and cerebellar damage, even if there has been initial "sparing" of the CNS.

Damage may cause cerebral palsy and developmental disabilities in later life. The fetus of a high-risk pregnancy may experience such damage before birth, with recovery of biochemical markers of distress, such as metabolic acidosis so that these are not apparent after birth [27]. If myocardial contractility is impaired following severe or sustained hypoxia, the resultant reduction in cardiac output may further compromise cerebral blood flow and other organ perfusion. Again, depending on the degree of insult, this can be associated with acute myocardial dilatation and consequent tricuspid regurgitation, myocardial ischemia, and hypotension.

Renal impairment is commonly reported following a generalised hypoxic-ischemic insult at birth. The degree of insult varies in effect from oliguria with minor electrolyte abnormality and minimally elevated creatinine, to complete renal failure requiring dialysis.

An elevated blood concentration of liver enzymes, as a marker of hepatocellular injury due to perinatal hypoxia, is also common after acute hypoxia, but irreversible liver damage is rare.

Fetal cardiovascular and endocrine responses may be altered, both in acute and in chronic hypoxia. Recurrence of mild hypoxia may occur in pregnancies where the blood flow to placenta, uterus, and fetus is repeatedly compromised by physiological and environmental influences. In chronic hypoxia, fetal growth restriction is not uncommon, and depression of growth factors during hypoxia has an important protective effect in conserving fetal substrate for energy as opposed to growth needs [28, 29]. The full-term infant, while more likely to survive a severe hypoxic-ischemic insult at birth than a preterm infant (approximately 70% *vs* 30%), is also more likely to have significant long-term morbidity [30].

2.7 Postnatal Development

2.7.1 Adaptation to Extrauterine Life

With the first breath after birth, changes in the cardiopulmonary system occur. The first challenge for a newborn is the provision of oxygen by independent breathing instead of utilizing placental oxygen. With the first breaths, there is a fall in pulmonary vascular resistance, and an increase in the surface area available for gas exchange. As the pulmonary vascular resistance falls, there is a corresponding increase in systemic vascular resistance due to loss of the low-resistance placental circulation. These two changes result in a rapid redistribution of blood flow to the pulmonary vascular bed from approximately 4% to 100% of the cardiac output, with an increase in blood oxygen delivery. The consequent increase in pulmonary venous return results in the left atrial pressure being slightly higher than the right atrial pressure, which closes the foramen ovale. This changed flow pattern results in decreased blood flow across the ductus arteriosus and the higher blood oxygen content stimulates the constriction and ultimately the closure of this fetal circulatory shunt. The umbilical vein and the ductus venosus close off within two to five days after birth, leaving behind the ligamentum teres and the ligamentum venosus of the liver, respectively.

These cardiovascular system changes result in a transition from fetal to adult circulation pattern. During this transition, some types of congenital heart disease that were not symptomatic in utero when there was a fetal circulation will present with cyanosis or respiratory signs (see Chapters 75 and 76).

2.7.2 The Preterm Fetus

In Europe and many developed countries, the preterm birth rate is generally 5–9%, and in the USA it has risen to 12–13% in the last decades. There are three categories of preterm birth: (1) spontaneous preterm births are the 40–45% preterm births that follow preterm labour of spontaneous (i.e. idiopathic) onset; (2) 25–30% preterm births occur after premature rupture of the membranes; (3) the remaining 30–35% are preterm births that are induced for obstetric reasons. Full-term pregnancy is from 37 to 41 completed weeks and babies born just a few weeks earlier usually do not experience any problems related to their slight prematurity. However, the more premature these infants are, the more serious are the complications.

The term intrauterine growth restriction (IUGR) describes a condition in which the fetus is smaller than expected for the number of weeks of pregnancy. Newborn babies with IUGR are often described as small for gestational age (SGA). A fetus with IUGR often has an estimated fetal weight below the tenth percentile and may be born at term (after 37 weeks of pregnancy) or prematurely (before 37 weeks). IUGR refers to a condition in which a fetus is unable to achieve its genetically determined potential size. This functional definition seeks to identify a population of fetuses at risk of poor outcome. The clinician's challenge is to identify IUGR fetuses whose health is endangered in utero because of a hostile intrauterine environment and to monitor and intervene appropriately. Increasingly, data support the notion that long-term consequences of IUGR last well into adulthood. These individuals are predisposed to the development of a metabolic syndrome later in life, manifesting as obesity, hypertension, hypercholesterolemia, cardiovascular disease, and type 2 diabetes. Several hypotheses suggest that intrauterine malnutrition results in insulin resistance, loss of pancreatic beta-cell mass, and an adult predisposition to type 2 diabetes. Although the causative pathophysiology is uncertain, the risk of a metabolic syndrome in adulthood is increased among individuals who were IUGR at birth [31]. In addition to an increased risk for physical sequelae, mental health problems have been found more commonly in children with growth restriction.

2.7.3 Diagnosis

Fetal arterial Doppler studies are useful in the differential diagnosis of SGA fetuses. In normal pregnancies, umbilical artery (UA) resistance shows a continuous decline as the pregnancy progresses; but this does not occur in fetuses with uteroplacental insufficiency.

The most commonly used measure of gestational age-specific UA resistance is the systolic-to-diastolic ratio of flow, the Pulsatility Index (PI), which increases with worsening disease. As the insufficiency progresses, end-diastolic velocity is lost and eventually reversed. The status of UA blood flow supports the diagnosis of IUGR and provides early evidence of circulatory abnormalities in the fetus, helping clinicians to identify these high-risk fetuses. UA Doppler measurements may help the clinician decide whether a small fetus is truly growth restricted and to identify a small fetus at risk of chronic hypoxemia. In hypoxemic fetuses with impaired placental perfusion, the PI in the umbilical artery is increased and the fetal middle cerebral artery PI is decreased; consequently, the PI ratio of the umbilical artery to middle cerebral artery (UA/MCA) is increased. However, the UA/MCA ratio does not appear to correlate significantly with outcome after 34 weeks.

Investigations of the venous vascular system have become increasingly important in the assessment of fetal myocardial function, and different indices are used to evaluate the blood flow velocity during the different phases of the cardiac cycle in the ductus venosus. Reference values for ductus venosus flow velocities are represented by ventricular systole (S wave) and diastole (D wave), the lowest forward velocity during atrial contraction (A wave). Different indices are calculated, e.g., the S/A ratio. The most important parameter which represents the final stage of disease is the abnormal reversal of blood flow velocities in the ductus venosus, inducing an increase in the S/A ratio, mainly due to a reduced A component of the velocity waveforms. Reference values should be used for ductus venosus flow velocities during ventricular systole (S wave) and diastole (D wave), the lowest forward velocity during atrial contraction (A wave) and different calculated indices as the S/A.

In the inferior vena cava, there is an increase of reverse flow during atrial contraction with progressive fetal deterioration, suggesting a higher pressure gradient in the right atrium. (Figs. 2.6 and 2.7). A high venous pressure induces a reduced velocity at end-diastole in the umbilical vein, causing typical

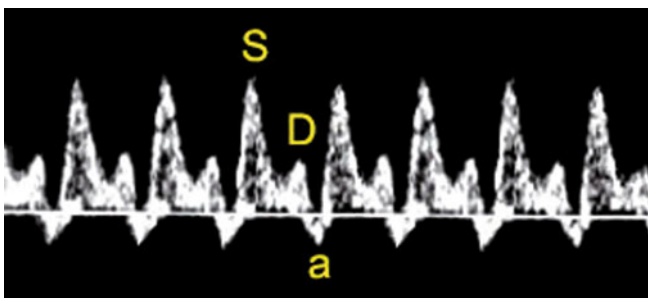

Fig. 2.6 Doppler examination of the inferior vena cava with normal flow velocity waveforms

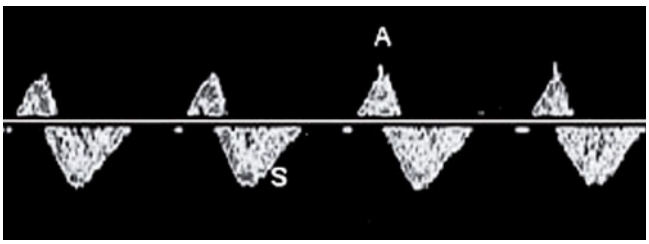

Fig. 2.7 Abnormal waveform with increase in reversed flow during atrial contraction in a growth-restricted fetus

end-diastolic pulsations. The development of these pulsations is close to the onset of abnormal fetal heart rate patterns and is frequently associated with acidemia and fetal endocrine changes. At this stage, there may be an increased coronary blood flow velocity compared with that seen in normally grown third-trimester fetuses and, if the affected fetus is not delivered, intrauterine death may occur within a few days.

2.7.4 Fetal Hemodynamic Aspects

Although the usual definition of preterm birth is birth before 37 weeks' gestation, a useful pragmatic definition for a "premature" infant is one who has not yet reached the level of fetal development that generally allows life outside the womb. In the normal human fetus, several organ systems mature between 34 and 37 weeks, and the fetus reaches adequate maturity by the end of this period. One of the main organs greatly affected by premature birth is the lung.

In umbilical venous blood, mild hypoxemia may be manifest through an absence of hypercapnia or acidemia. In severe uteroplacental insufficiency, the fetus cannot compensate hemodynamically, and hypercapnia and acidemia increase exponentially [32]. Hypoxemic growth-restricted fetuses also demonstrate a range of hematological and metabolic abnormalities, including erythroblastosis, thrombocytopenia, hypoglycemia, deficiency in essential amino acids, hypertriglyceridemia, hypoinsulinemia and hypothyroidism. Low birth weight increases the risk for perinatal mortality (death shortly after birth), asphyxia, hypothermia, polycythemia, hypocalcemia, immune dysfunction, neurologic abnormalities, and other long-term health problems [33].

2.7.5 Timing of Delivery and Management

In August 2004, *The Lancet* published data on brain development in survivors of the multicenter Growth Restriction Intervention Trial (GRIT) [34]. The aim of this study was to identify compromised fetuses between 24 and 36 weeks' gestation and answer the question of whether it was safer to deliver them immediately or to delay until there was no clinical doubt that delivery was necessary. Five-hundred and eighty-eight such fetuses were identified in 69 hospitals in 13 European countries. In the GRIT study, the 24 week gestation babies were very different from those at 36 weeks. In the absence of severe congenital abnormalities, the current infant mortality after 32 weeks' gestation is low: the causes of this rare event include asphyxia, necrotising enterocolitis and infection; respiratory distress syndrome is rare in this group. By contrast, before 32 weeks, and particularly in the extreme preterm fetus, there is a much higher mortality, and the levels of morbidity were recently emphasised by the EPICure Study, in which 49% of surviving infants born at less than 26 weeks' gestation had some disability at 30 months of age and 19% were severely disabled [35]. The EPICure study reached some important conclusions. It demonstrated that 44% of infants born at 25 weeks' gestation survived to discharge, whereas delivery at 22 weeks almost invariably resulted in neonatal death.

Neonatologists, obstetricians and parents must increasingly recognise that infants born less than 25 weeks' gestation who survive are at risk of disability at school age. In the EPICure study, only 20% were totally free of disability at school age and so the prognosis must be guarded. Disability was classified as follows:

1. *Severe*: the child was likely to be highly dependent on care-givers, e.g., non-ambulant cerebral palsy, profound hearing loss or blindness.
2. *Moderate*: children who were likely to be reasonably independent, e.g., ambulant cerebral palsy, some hearing loss, some visual impairment.
3. *Mild*: children with neurological signs with minimal functional consequences.

In the EPICure study, over half of the survivors had moderate disability or no disability at school age. In addition, some of the 24% with moderate disability were improved with spectacles and hearing aids.

There is uncertainty about whether iatrogenic delivery of the very preterm (before 33 weeks of gestation) growth restricted fetus should be undertaken before the development of signs of severe hypoxemia, with a consequent risk of prematurity-related neonatal complications, or whether delivery should be delayed, incurring risks of prolonged exposure to hypoxia and malnutrition imposed by the hostile intrauterine environment [36]. With every week that passes, there is a decreasing risk of complications including intraventricular hemorrhage, retinopathy of prematurity and sepsis. However, delay may expose the growth-restricted fetus to ischemic injury of the brain, resulting in asphyxia, periventricular leukomalacia and intraventricular hemorrhage, as well as a significant risk of intrauterine death. It is important to weigh the risks and benefits of early interventions. This is a dynamic process, in which advancements in both fetal and neonatal medicine are of crucial importance for the appropriate counselling of parents and the management of these pregnancies.

The GRIT study showed a small increase in fetal death if the obstetrician delayed delivery, and a small increase in

Table 2.2 Suggested management of the preterm fetus

What to do	Perform parental counselling Share any type of decisions with the neonatologist, the anesthesiologist and the couple, personalizing the specific situation Fill the informed consent as much detailed as it is possible
Considering	Short-term consequences: RDS, NEC, IVH, PVL, pulmonary dysplasia, sepsis Long-term consequences: cerebral palsy, mental impairment, attention disorders Pregnancy age and prognosis age Etiology of the preterm labour (maternal causes, fetal causes) Maternal mortality related to the type of delivery Fetal presentation Obstetric anamnesis of the patient Combination of the multiple factors
When	Better after 26 weeks Using corticosteroids between 48 hours and 7 days before delivery
Where	Any hospital with NICU
How	Trying to reduce the effects of the hypoxia Balance maternal and fetal morbidity Preterm delivery is not itself an indication of cesarean section unless associated with maternal or fetal consequences

neonatal death if early delivery was chosen. Thus the monitoring of fetal health is particularly important if there is growth restriction. Such fetuses have few metabolic reserves, and sudden death during pregnancy may occur. Labor is an intermittently hypoxic event, and anaerobic metabolism may not be an option when there are inadequate stores of fat and glycogen.

In recent years, placental and fetal arterial Doppler flow-velocity waveforms have guided the timing of delivery. Doppler has been particularly effective in assessing the growth-restricted pregnancy and has been a useful adjunct for the assessment of the very preterm fetus, when cardiotocographical monitoring is unhelpful. However, in the growth-restricted hypoxemic fetus, redistribution of well-oxygenated blood to vital organs, such as the brain, heart and adrenals, represents a compensatory mechanism to prevent fetal damage, and when the reserve capacities of the circulatory redistribution reach their limits, fetal deterioration may occur rapidly. In clinical practice, serial Doppler investigations estimate the duration and degree of fetal blood flow redistribution. The onset of an abnormal venous Doppler recording indicates deterioration in the fetal condition and iatrogenic delivery should be considered.

In conclusion, the goal in the management of the preterm fetus is to deliver the most mature fetus possible, at least at 32–34 weeks, in the best condition possible while minimizing the risk to the mother (Table 2.2). There is lack of a firm evidence base and IUGR fetuses remain a challenging problem for clinicians. Most cases of IUGR occur in pregnancies in which no risk factors are present and the clinician must therefore be alert to the possibility of growth disturbance in all pregnancies. No single measurement secures the diagnosis and a complex strategy for diagnosis and assessment is therefore necessary. The current therapeutic goals are to optimize the timing of delivery to minimize hypoxemia and maximize gestational age and maternal outcome.

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3

General Characteristics of Preterm and Term Newborn

Stefano Parmigiani, Daniela Gianotti and Giulio Bevilacqua

The aim of both parents and healthcare professionals is to achieve a normal healthy infant. This chapter deals with some general principles.

3.1 History

A full family history is essential. This should include a full medical and social history. Note should be taken of alcohol ingestion and of any drugs (prescribed or recreational). It should enquire about the possibility of consanguinity – the question, "Are your families related?" to both parents is one way of approaching this often delicate subject. Enquiry should be made about the presence of possible transmissible and inheritable diseases in the families of both parents. Tall or short stature can generate a search for specific undiagnosed diseases in the parents (e.g., Marfan syndrome, gluten intolerance, achondroplasia). Anemia in the parents can be a marker of a hematological defect (e.g., thalassemia), as well as the place of origin and ethnicity of the parents (e.g., G6PD deficiency).

3.2 History of the Pregnancy and Delivery

A baby's well-being is determined by periconceptional events. The mother's medical history is important, including the possibility of maternal diabetes mellitus and other illnesses and her immune status (HBV, HCV, HIV, CMV, toxoplasmosis, rubella, HSV-HZV, and syphilis). The possibility of seroconversion during pregnancy should be considered. Enquiry should be made about the course of the pregnancy. Account should be taken of the time of booking for antenatal care (late booking may be a sign of a disorganized life style and associated problems).

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The results of antenatal checks should be noted: fetal growth and ultrasound results, amniotic fluid volume, maternal anemia, urine results and maternal diabetes mellitus, pregnancy-induced hypertension or pre-eclampsia. The results of vaginal or anal bacterial swabs within the month before delivery should be noted (group B streptococci or *Listeria monocytogenes*) and whether the mother was given appropriate intra-partum antibiotic prophylaxis.

A history of the pregnancy should include note of drugs taken during the pregnancy and their indications. Consider evidence of infectious illnesses or fever close to the time of delivery and take note of the timing of membrane rupturing and the quantity and color (blood or meconium staining) of the amniotic fluid.

Details of the delivery should be noted, i.e., whether vaginal, operative (forceps of vacuum extraction), cesarean section (planned or emergency, before or during the labor), and evidence of fetal distress.

The baby's presentation should be noted because abnormal limb position may be due to a breech presentation. The possibility of birth trauma (e.g., cephalhematoma, fracture of the clavicle) should be considered.

Adaptation to postnatal life should be considered, bearing in mind that during the first hours after birth the baby is in a transitional period, passing from intra- to extrauterine life.

A baby's condition during the minutes just after birth is described by the Apgar score, usually recorded at 1 and 5 minutes (see Table 3.1). Although the Apgar score may be criticized (it is subjective on the part of the observer, often recorded some time after delivery), in its favor, it is almost universally recorded. The score was described in 1953 by Dr Virginia Apgar, a North American pediatric anesthetist. She intended it to indicate whether or not resuscitation was needed. Although imperfect, there is no doubt that a low score (0 or 1, signifying an absent slowly beating heart) indicates a baby who is barely alive at the time of birth, and a score of 8 or more indicates an individual whose general condition is good. However, the Apgar score is an imperfect indicator of subsequent progress or outcome. A baby who has

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 3.1 Apgar Score

Sign	0	1	2
Appearance (colour)	Blue or pale	Pink body with blue extremities	Completely pink
Pulse (heart rate)	Absent	< 100 beats/min	> 100 beats/min
Grimace (reflex irritability)	No response	Grimace	Cough or sneeze
Activity (muscle tone)	Limp	Some flexion	Active movements
Respiration	Absent	Slow, irregular	Good, crying

a high initial score may develop difficulties with gas exchange in the minutes that follow, even if these problems are transient.

Most healthy babies are inclined to breastfeed during the first hour of life, and babies recognize their mother's smell.

A baby's size is an indicator of intrauterine development and nutrition. Intrauterine growth restriction can be a consequence of poor placental function (e.g., due to diseases such as pregnancy-induced hypertension, systemic lupus erythematosus, cocaine ingestion, infection) and congenital disease, chromosomopathy, or fetal alcohol syndrome.

Observation of a baby's movements when undressed and preferably in the presence of one or both parents, allows for observation of the quality of movements (whether symmetrical, coordinate and smooth).

Eyes may be difficult to examine at birth because there is often some eyelid edema, but they must be checked later to note the presence of a red reflex. In the case of babies who are discharged early from hospital, this may need to be done at home by the family doctor or community midwifery team.

Lips, gums and palate must be examined to exclude the presence of a cleft or other malformation.

The hips should be examined to exclude congenital dislocation. During the immediate neonatal period, the ability to achieve full abduction is a useful sign. It may only be possible to elicit a positive Ortolani sign, an index of hip luxation/subluxation, later during the first week.

Arms, legs, fingers and toes should be examined to identify any abnormality (e.g., webbing, number, length). Examination of the chest should note any asymmetry during respiration, subcostal or intercostal retraction, ancillary nipples. The skin may show areas of hypo- or hyper- pigmentation, scars, blisters, pustules, petechiae, or evidence of birth trauma (cuts, bruises). The diaper area should be examined to look for anomalies of the external genitalia, an imperforate or anterior anus, and hairy tufts or dimples over the sacrum.

3.3 Clinical Aspects of CNS Development

Motor and sensory functions mature during pregnancy and are already in great part developed by the middle of the second trimester of pregnancy and the process continues after birth. Ultrasound, particularly so-called 4D ultrasound, allows for the identification and classification of fetal movements, and natural fetal behavior has been studied. Sporadic, irregular ("vermiform") movements are seen between 6 and 7 weeks' gestation, involving the whole body. Generalized, brief movements from the legs to the neck and head (such as "startle") are observed at 8 weeks' gestation. At 9 weeks, flexor movements of the cranial and caudal poles towards the centre appear, interspersed with jerks that allow small movements within the amniotic liquid and partial rotation of the head. At the tenth week, hand-to-head movements, mouth opening with tongue protrusion, swallowing, rotation along the longitudinal axis and independent movements of limb flexion and extension can be observed [1, 2].

The maturation of the central nervous system (CNS) determines an infant's response and tolerance to various sensory inputs. A premature infant may demonstrate signs of immaturity [3] such as:

- Diffuse and indeterminate sleep or waking states with frequent whimpering, facial twitches or apparent smiling
- Abrupt transitions between states
- Periods of fussiness or crying
- Low-level alertness, characterized by a dull, glassy-eyed look
- Hyper-alertness, characterized by wide-open eyes with a panicked, worried look; an appearance of extreme vigilance
- Uncoordinated eye movements: roving or floating eyes
- Immature tone, posture and general coordination.

The five senses can be altered by prematurity:

1. The earliest response to auditory stimulation has been recorded at 19 weeks' gestation, but consistent responsiveness is established by 25 weeks. Deafness is a complication of intraventricular hemorrhage or periventricular leukomalacia affecting 5–10% of preterm infants. It may also be the result of a cytomegalovirus infection, which may have caused preterm delivery. The ambient noise of a NICU is rarely less than 40 dB (as in the womb), and more often around 70–100 dB [4].
2. Functional maturation of the visual system starts at around 5 months and it is still immature at term [5]. Retinopathy of pre-maturity (ROP), formerly known as retrolental fibroplasia and affecting premature babies exposed to excessive oxygen, is nowadays largely preventable: oxygen delivery is monitored carefully and all infants below 32

weeks' gestation have regular examination of the retina following a generally accepted international protocol. Advanced stages of ROP are treated by cryo- or laser- therapy. However, decreased visual acuity or strabismus may follow. Nowadays, total blindness is infrequent.

3. Taste develops under the influence of amniotic fluid flavonoids and odorants transmitted from the maternal diet from as early as 14 weeks' gestation. Taste after birth can be disturbed by drugs, by the late introduction of oral feeding, or by the impaired development of taste centers.
4. The sensory system starts to develop at 8 weeks and is functional by 12 weeks' gestation. Being touched may be uncomfortable even for full-term infants who have not developed all the receptors and pathways, and perception may also be disturbed by drugs, e.g., maternal cocaine use. In the preterm infant, touch may be a powerful stressor. Touch should be gentle and combined with the stimulation of other senses, for example, speaking to the baby. Touching for no good reason should similarly be avoided. Parents should be taught to touch gently and about "kangaroo care".
5. The olfactory tract is part of the primitive encephalon and a baby at term is able to recognize its mother's odor. The olfactory system is fully functional by 14 weeks' gestation. Strong odors can be stressful for preterm babies who are unable to communicate this to their carer.

These signs of immaturity should be recognized and a favorable environment should be created, avoiding overstimulation. Woolf [6, 7], Brazelton [8] and Prechtl [9] described different states of behavior. The Brazelton scale assesses neonatal behavior in six states: quiet sleep, active sleep, drowsiness, alert inactivity, active awake, crying (Table 3.2). The baby should be observed in a state of quiet wakefulness.

3.4 The Preterm Infant

Maturity is determined by the length of gestation, and the severity of problems related to pre-maturity are directly related to gestation.

Weeks of gestation are generally considered as completed weeks. The World Health Organization has defined preterm infants as those with gestational age less than 37 weeks. Recently, the term "late preterm infants" (instead of "near-term") has been used for those infants that are born at a gestational age between 34 weeks, and 36 weeks and 6 days. These infants have a rather higher morbidity and mortality than term infants (gestational age ≥ 37 weeks), even though they are of similar size [6–9]. Some North American authors have also used the terms "premies" and "micropremies" to describe very immature babies.

Classification by birth weight is as follows:

- *low birth weight* (LBW), 1501–2500 g;
- *very low birth weight* (VLBW), 1001–1500 g;
- *extremely low birth weight* (ELBW), ≤ 1000 g.

Because of the survival of very light and premature babies, the term "incredibly low birth weight" has been used to refer to babies weighing less than 750 g.

A fundamental problem for all preterm infants is their poor ability to maintain body temperature, because of reduced glycogen stores (depending on gestational age) and thinner skin, with the most immature lacking the ability to shiver. Thus a primary aim is to avoid heat loss (and insensible water losses) by drying, heating, and covering the baby. This also decreases glucose consumption, reducing the risk of hypoglycemia.

The preterm baby often experiences delayed respiratory adaptation. Depending on the degree of immaturity, the lungs

Table 3.2 Neonatal states classification scale

State	Characteristics
Quiet sleep	Regular breathing, eyes closed. Spontaneous activity confined to startle and jerky movements at regular intervals. Responses to external stimuli are partially inhibited, and any response is likely to be delayed. No eye movements, and state changes are less likely after stimuli or startles than in other states.
Active sleep	Irregular breathing patterns, sucking movements, eyes closed but rapid eye movements can be detected underneath the closed lids. Infants also have some low-level and irregular motor activity. Startles occur in response to external stimuli and can produce a change in state.
Drowsiness	While the newborn is semi-dosing, eyes may be open or closed; eyelids often flutter; activity level variable and interspersed with mild startles. Drowsy newborns are responsive to sensory stimuli but with some delay, and state change frequently follows stimulation.
Alert inactivity	A bright alert look, with attention focused on sources of auditory or visual stimuli; motor activity is inhibited while attending to stimuli.
Active awake	Eyes open, considerable motor activity, thrusting movements of extremities, and occasional startles set off by activity; reactive to external stimulation with an increase in startles or motor activity. Discrete responses are difficult to distinguish due to general high activity level.
Crying	Intense irritability in the form of sustained crying, and jerky limb movement. This state is difficult to break through with stimulation.

Data from [8].

are morphologically immature and lack surfactant. Such babies may require the endotracheal administration of exogenous surfactant and mechanical ventilation. Bronchopulmonary dysplasia (chronic lung disease with O_2 dependency) is a complication of severe prematurity, which may continue to cause problems during subsequent years.

Premature infants have reduced immune defences. Furthermore, infection may be the primary cause of preterm delivery, sometimes affecting the baby before birth. Such infections, in combination with lung and brain immaturity, increase the risk of later disability (see Chapter 18).

The gastrointestinal tract is not yet adapted to enteral feeds, posing considerable challenges to those responsible for their care. Early non-nutritive feeding should be considered. Milk, preferably from the mother or a human milk bank may be started early but cautiously, particularly for the most immature infant. Careful note should be taken of early signs of gastrointestinal intolerance, e.g., increased volume or bile-staining of gastric aspirates or abdominal swelling. The most immature infants require parenteral nutrition (partial or total) to provide adequate nutrients and calories for growth, and this may need to be continued for several weeks. This practice requires a central, indwelling catheter, increasing the risk of infection.

Because of advances in the care of these very vulnerable infants, survival at the earliest gestation is improving.

Survival at early gestation and the associated risks of neurodevelopmental impairment are considered elsewhere (see Chapter 21).

Not only babies at extremely low gestation, but also those born late preterm are at risk.

Various evidence points to the environment being of major importance for appropriate development. Although neonatologists strive to recreate an extrauterine environment that is similar to that of the womb, there are many differences.

Light, painful interventions, development in air instead of surrounded by amniotic fluid, noise, stress, sleep-wake cycles interrupted by nursing procedures, continuous intravenous nutrition (without the intermittent glycemic peaks of normal feeding and maternal ingestion), fluctuations in oxygen delivery, carbon dioxide and pH levels and blood pressure may all interfere with normal brain development. A high tech/soft touch approach may be beneficial and a mother's touch and breastfeeding should be encouraged even for very tiny babies.

In some units, parents are supported by a psychologist to help with attachment. In spite of the best endeavors of staff, having a baby in a neonatal unit, often for several months, is undoubtedly stressful, giving rise to feelings of inadequacy on the part of the mother, who may feel a biological failure, and anxiety on the part of the father, who is no longer in control of the situation.

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4

Risk Factors for Gestational Diseases

Maria De Bonis, Michela Torricelli and Felice Petraglia

4.1 Introduction

Preterm delivery (PTD) and pre-eclampsia (PE) are severe pathologies that may affect and compromise the course of pregnancy, and adverse pregnancy outcomes are important causes of perinatal morbidity and mortality [1].

Interventions to reduce the morbidity and mortality related to PTD and PE can be classified as primary (detected in all women before or during pregnancy to prevent and reduce risk); secondary (aimed at eliminating or reducing risk in women with known risk factors); or tertiary (initiated after labor has begun, with a goal of preventing delivery or improving outcomes for preterm infants).

Most obstetric interventions to reduce morbidity and mortality are classified as tertiary, but the primary prevention of prematurity-related illness is desirable.

4.2 Preterm Delivery

PTD, defined as birth before 37 weeks' gestational age, occurs with an incidence of 7–11% and represents one of the predominant causes of perinatal mortality and morbidity [2]. Very PTD is birth before 32 weeks and extremely preterm delivery before 28 weeks of gestation [3]. PTD is a syndrome initiated by various conditions, such as inflammation/infection, uteroplacental ischemia or hemorrhage, uterine overdistension, cervical disease, stress and endocrine disorders, and other immunologically mediated processes [4]. Several risk factors cause a transition from uterine quiescence to PTD, which may be associated with preterm premature rupture of membranes (PPROM). Many of the risk factors are associated with increased systemic inflammation, and this pathway might explain some of the increases in PTD associated with multiple risk factors [5].

4.2.1 Risk Factors for Preterm Delivery

Various maternal and fetal characteristics have been associated with PTD. These include maternal demography (e.g. poor socio-economic status, ethnicity, age), poor nutritional status, pregnancy history, characteristics of the present pregnancy, and associated conditions (e.g., smoking, alcohol ingestion, drugs of abuse (particularly cocaine and opiates), infection, uterine contractions and cervical length, and biological and genetic markers (Table 4.1) [6].

Table 4.1 Risk factors for preterm delivery

Preconceptional
– Socio-economic characteristics
– Previous preterm delivery
– Interval between pregnancies
– Nutritional status
Maternal disorders
– Systemic diseases
– Local or systemic infections
– Previous uterine surgery
External factors
– Stress
– Smoking
– Drugs of abuse
Pregnancy-associated risk factors
– Multiple pregnancy
– Intrauterine infection
– Vaginal bleeding
– Bacterial vaginosis
– Cervical shortening and insufficiency
Biochemical and genetic markers
– Fibronectin, CRH

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4.2.1.1 Preconceptional Factors

- *Socio-economic characteristics*: Black women are three times more likely to have a very early PTD than women from other ethnic groups [7]. Low socio-economic status, low and high maternal age, and being unmarried are also associated with PTD [8].
- *Previous PTD*: The recurrence risk in women with a previous PTD ranges from 15–50%, depending on the number and gestational age of previous deliveries. The risk of another PTD is inversely related to the gestational age of the previous PTD [9].
- *Interval between pregnancies*: An interpregnancy interval of less than 6 months confers a greater than two-fold increased risk of PTD [10].
- *Nutritional status*: A low pregnancy body-mass index (BMI) is associated with high risk of spontaneous PTD. Women with low serum concentrations of iron, folate, or zinc have more PTD than those with measurements within the normal range [11].

4.2.1.2 Maternal Disorders

- *Systemic diseases*: Thyroid diseases, asthma, diabetes and hypertension, are associated with increased rates of PTD.
- *Local or systemic infections*: Infections, such as pyelonephritis and asymptomatic bacteriuria, pneumonia and appendicitis, are associated with PTD [12]. Periodontal disease appears to have an increased risk independent of other factors [13].
- *Previous uterine surgery*: History of myomectomy, cervical cone biopsy sample or loop electrocautery excision procedures have been associated with an increase in spontaneous PTD [14].

4.2.1.3 External Factors

- *Stress*: Mothers experiencing psychological stress are at increased risk of PTD. Although the mechanism underlying the link between psychological or social stress and increased risk of PTD is unknown, a role has been proposed for corticotropin-releasing hormone [15].
- *Smoking*: Tobacco use increases the risk of PTD. Both nicotine and carbon monoxide are powerful vasoconstrictors, and are associated with placental damage and decreased uteroplacental blood flow, leading to fetal growth restriction and PTD [16].
- *Drugs of abuse*: Several maternal drugs affect the developing fetus and the baby after birth may manifest signs attributable to withdrawal. Maternal cocaine, opiate and diazepam use are particularly relevant, as is a history of excessive alcohol ingestion, especially binge drinking.

4.2.1.4 Pregnancy-Associated Risk Factors

- *Multiple pregnancy*: Multiple gestations carry a risk of PTD, and result in 15–20% of all PTD. Uterine overdistension, resulting in contractions and PPROM, is believed to be the causative mechanism for the rate of increased spontaneous PTD [16].
- *Intrauterine infection*: Intrauterine infection is an important mechanism leading to PTD. The mechanisms by which intrauterine infection lead to PTD are related to activation of the innate immune system. Microbial endotoxins and proinflammatory cytokines stimulate the production of prostaglandins and matrix-degrading enzymes. Prostaglandins stimulate uterine contractility, whereas degradation of the extracellular matrix in the fetal membranes leads to PPROM [4]. The microorganisms most commonly reported in the amniotic cavity are genital *Mycoplasma* [17].
- *Vaginal bleeding*: Vaginal bleeding caused by placental abruption or placenta praevia is associated with a very high risk of PTD, but bleeding in the first and second trimesters that is not associated with either placental abruption or placental praevia is also associated with subsequent PTD [18].
- *Bacterial vaginosis*: The mechanism by which bacterial vaginosis is associated with PTD is unknown, but microorganisms that cause the infection probably ascend into the uterus before or early during a pregnancy [19].
- *Cervical shortening and insufficiency*: During labor, the cervix shortens, softens, rotates anteriorly, and dilates. Both digital and ultrasound examinations of the cervix have shown that cervical shortening is a risk factor for PTD. The shorter the cervix, the greater the risk (<2.5 cm by vaginal ultrasound) [20].

4.2.1.5 Biochemical and Genetic Markers

Biological fluids (amniotic fluid, urine, cervical mucus, vaginal secretions, serum or plasma and saliva) have been used to assess the value of biomarkers for the prediction of PTD. Cytokines, chemokines and estriol are associated with PTD. The most useful biochemical PTD predictor is fetal fibronectin, a glycoprotein that when present in cervicovaginal fluid is a marker of choriodecidual disruption [21]. Although fetal fibronectin is normally absent from cervicovaginal secretions from 24 weeks until term, 3–4% of women undergoing routine screening at 24–26 weeks are positive, and at increased risk of PTD. A gene-environment interaction has been shown with maternal carriage of an allele of the tumor necrosis factor α (TNFα) gene and bacterial vaginosis. Genetic factors could also be involved for stress mediators of the maternal and fetal hypothalamic-pituitary axis, such as corticotropin-releasing hormone (CRH), prostaglandins and

oxytocin [22]. Several studies support the involvement of placental CRH in the mechanisms controlling the onset of labor. Maternal serum CRH concentrations are similar to the CRH curve of normal pregnancy, but with a higher level [23].

4.3 Pre-eclampsia

PE is defined as persistent blood pressure elevation, edema, and proteinuria first diagnosed after 20 weeks of gestation. The minimal criteria for the diagnosis of PE are proteinuria, defined as 300 mg or more of urinary protein excretion per 24 h, and hypertension, defined as blood pressure of 140/90 mmHg or higher [24]. PE affects 5–7% of all pregnancies and is responsible for 7–15% of maternal mortality. Severe and/or early-onset PE is an important cause of fetal and maternal morbidity and mortality. Neonatal outcomes are directly related to iatrogenic prematurity, although uteroplacental insufficiency may also be associated with the disease. The only known cure is cessation of pregnancy, although temporizing measures are often undertaken [25]. During endovascular cytotrophoblast invasion in the spiral arteries, an exaggerated inflammatory response and inappropriate endothelial cell activation are key features in the pathogenesis of PE.

4.3.1 Risk Factors for Pre-eclampsia

Several studies identified risk factors for early detection of PE (Table 4.2) [26].

Table 4.2 Risk factors for pre-eclampsia

Preconceptional
- Nulliparity
- Previous pre-eclampsia
- Family history

Maternal disorders
- Chronic hypertension and renal disease
- Obesity, insulin resistance and gestational diabetes
- Thrombophilic disorders

External factors
- Stress

Pregnancy-associated risk factors
- Multiple pregnancy
- Congenital and chromosomal anomalies

Biophysical markers
- Uterine artery Doppler sonography

Biochemical markers
- hCG, Activin A, Inhibin A, PIGF and VEGF, PAPP-A

4.3.1.1 Preconceptional Factors

- *Nulliparity*: As consequence of an abnormal response to paternal antigens in fetoplacental unit.
- *Previous pre-eclampsia*: Mothers who had PE in their first pregnancy are at higher risk for the development of PE in a subsequent pregnancy, especially when PE is severe, occurs early during the pregnancy, or is associated with a low birth weight [27].
- *A family history of pre-eclampsia*: Severe PE and eclampsia have a familial occurrence. A family history of PE is associated with a three-fold increased risk of PE and a four-fold increased risk of severe PE.

4.3.1.2 Maternal Disorders

- *Chronic hypertension and renal disease*: The risk of developing superimposed PE is particularly increased in women with severe hypertension and in those with cardiovascular or renal disease. The incidence of superimposed PE increases in patients with chronic renal disease, especially if there is coexisting hypertension.
- *Obesity, insulin resistance, and gestational diabetes*: Obesity, insulin resistance, and glucose intolerance are strongly associated with non-pregnant hypertension. Essential hypertension is an insulin-resistant state itself. Obesity is probably the most common cause of insulin resistance and represents a risk factor for developing pregnancy-induced hypertension as well as PE [28]. There is a certain degree of insulin resistance and hyperinsulinemia in pre-eclamptic women during pregnancy. The insulin resistance appears to be based on a higher mean body mass index in women with PE. Overt type 1 diabetes mellitus is associated with an increased incidence of PE [25].
- *Thrombophilic disorders*: Patients with severe early-onset PE often have hemostatic or metabolic abnormalities, which are associated with a tendency to vascular thrombosis. There is an increased incidence of activated protein C (aPC) resistance or factor V Leiden mutation in women with a history of PE and/or adverse perinatal outcome [30]. Thus women with familial thrombophilia are at increased risk not only of PE, but also of fetal loss. Protein S deficiency and aPC resistance both result in an impaired aPC patway. This impairment appears to be associated with a more aggressive course of the pathologic changes (thrombosis, acute atherosis) in the spiral arteries [31]. Classic homocystinuria is the homozygous form of the autosomal recessively inherited cystathionine-β-synthase deficiency. The incidence of hyperhomocysteinemia increases the risk for pre-eclampsia, mainly for severe early-onset PE [32].

4.3.1.3 External Factors

- *Stress*: Working women have 2.3 times the risk of developing PE compared with non-working women [33].

4.3.1.4 Pregnancy-Associated Risk Factors

- *Multiple pregnancy*: The incidence and severity of PE, the incidence of eclampsia, and the incidence of early-onset PE are significantly increased in patients with twin pregnancies, who have a four-fold increased risk of PE [34].
- *Congenital and chromosomal anomalies*: Malformations of the male genital apparatus should be considered as risk factors for PE [35]. There is also an increased incidence of pre-eclampsia in cases of hydrops fetalis. Trisomy 13 can be associated with PE, supporting the argument for a fetal factor in the pathogenesis of PE [36].

4.3.1.5 Biophysical Markers

Reduced uteroplacental blood flow and placental ischemia leads to the release of placental factors with detrimental effects on the maternal vascular endothelium, leading to a rise in blood pressure and changes in the uterine artery flow velocities. In the early days, the most important predictors of PE were those related to the early recognition of raised blood pressure in the mother; more recently, there has been detection of impaired uterine artery blood velocities and particularly their wave forms by uterine artery Doppler sonography (notch) [37]. Assessed during midtrimester, such abnormal Doppler findings indicate a substantially increased risk for the development of PE [38]. Uterine artery Doppler sonography more accurately predicted PE than intrauterine growth restriction and the most powerful Doppler index for predicting PE was an increased pulsatility with notching in the second trimester.

4.3.1.6 Biochemical Markers

Trophoblastic abnormalities play a central role in the development of PE and precede the appearance of clinical signs and symptoms; some placental hormones change in the maternal circulation, indicating altered placental function. The levels of several placental hormones are elevated in maternal serum before the diagnosis of PE, and these may be considered preclinical manifestations of the earlier stages of the disease. Such hormones have therefore been proposed as early predictive markers of PE [23]. Women with PE in the third trimester have increased maternal serum human chorionic gonadotropin (hCG) levels. Maternal hCG levels are already increased in the second trimester in pregnancies that subsequently develop PE [39].

Maternal serum activin A and inhibin A levels are increased in the presence of hypertensive disorders [40, 41]. Because activin A is involved in the control of trophoblast cell differentiation in the first trimester [42], altered expression of this protein may affect placental invasiveness, resembling the pathogenesis of PE. Inhibin A is elevated several weeks before the onset of clinical signs of PE [39, 43]. However, when both proteins are measured at 15–19 weeks', inhibin A appears to be more sensitive than activin A in predicting early-onset PE culminating with delivery before 34 weeks [43].

Phosphatidylinositol-glycan biosynthesis class F (PlGF) and vascular endothelial growth factor (VEGF) are potent angiogenic factors, inhibited by the soluble form of the vascular endothelial growth factor receptor-1 (sVEGFR-1), also known as soluble fms-like tyrosine kinase-1 (sFlt-1), which may be considered the most important candidate for preventing of PE and also targets placental growth factor. PlGF and VEGF-A are expressed by trophoblasts and show altered expression patterns in PE, and particularly lower PlGF. sFLT-1 serum concentrations are also increased in PE and correlate with the severity of the disease [44].

Pregnancy-associated plasma protein A (PAPP-A) is a large glycoprotein complex, which is predominantly produced by the placenta. PAPP-A cleaves insulin-like growth factor binding protein (IGFBP)-4 and -5 [45] and, thereby, modulates the activity of insulin-like growth factor (IGF)-1 and -2. Moreover, PAPP-A has been suggested to play a role in human implantation [46]. Its circulating levels were found to be elevated after the onset of PE and lower in women who subsequently developed PE [47].

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5

Epigenetic Mechanisms

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5.1 Introduction

While phenotypic variation such as disease vulnerability has traditionally been viewed as being determined by the interaction between genes and the environment, it is now clear that this is over-simplistic. *Developmental plasticity* describes the phenomenon whereby development of the phenotype from a given genotype is influenced by developmental experiences, and phenotypic variation in turn influences how the individual interacts with its mature environment, thus affecting disease risk. It is now recognized that developmental plasticity is underpinned by epigenetic processes, which are environmentally-induced changes in the patterns and regulation of gene expression brought about by a set of modifications in DNA and DNA-associated molecules, without changes in the base sequence. Epigenetic processes are phylogenetically old, and the mechanisms involved modulate both gene dosage and the conditions under which genes are expressed. Developmental plasticity is but one of several processes which are effectuated by epigenetic mechanisms; in mammals other processes include transposon silencing, cell differentiation, X-inactivation in females and genomic imprinting.

This chapter describes several of the most extensively studied epigenetic mechanisms to date. Their involvement in the development of metabolic and cardiovascular diseases, which stem from maladaptive fetal responses; and imprinting disorders, which result from defects in the establishment or erasure of specific epigenetic marks, is discussed.

5.2 Epigenetic Mechanisms

Epigenetics, as used in the context of this chapter, refers to the molecular mechanisms that give rise to stable, mitotically heritable changes in gene function without a corresponding change in the DNA sequence. They include chromatin remodeling through histone modifications, DNA methylation, post-transcriptional control by regulatory RNAs, and genomic imprinting.

The genome in early life appears susceptible to environmentally induced epigenetic changes that can have persistent effects on structure and function.

5.2.1 Histone Modifications

In eukaryotes, DNA is wound around an octameric complex of histone proteins – two subunits each of the histones H2A, H2B, H3 and H4, forming the nucleosome (Fig. 5.1a). Chromatin is formed when nucleosomes are packaged closely, strung together by double-stranded DNA (Fig. 5.1b). Therefore, certain covalent modifications such as acetylation, methylation, phosphorylation, sumoylation, ADP ribosylation and glycosylation to specific amino acid residues on histone proteins can cause higher order structural changes. This remodeling of chromatin can influence gene expression depending on whether the modifications result in chromatin condensation or unwinding [1].

Histone modifications often occur on lysine residues at the amino-termini of histone tails. Lysine residues are commonly changed via histone modifying enzymes such as histone acetyltransferases (HAT), histone deacetylases (HDAC), methyltransferases, kinases and ubiquitilases. The most common modifications are acetylation, which involves addition of CH_3OH in a reaction catalyzed by HAT, and methylation, which involves addition of CH_3 (Fig. 5.1c).

Generally, increased acetylation leads to an increase in transcriptional activity (so-called active chromatin; see Fig. 5.3) whereas deacetylation, catalyzed by HDAC, leads to transcriptional repression (inactive chromatin) [2]. Some histone marks may have opposing effects of gene expression depending on the residue modified; trimethylation of the ninth lysine residue on histone H3 (H3K9me3) favors gene

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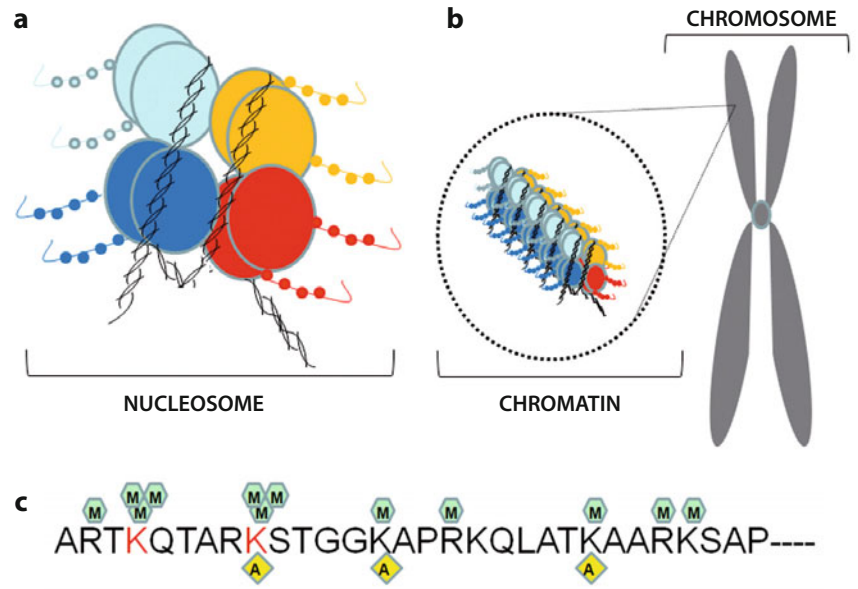

Fig. 5.1 **a** DNA is wound around an octameric complex of histones (comprising two subunits each of the four histones H2A, H2B, H3 and H4) to form the nucleosome. The amino-terminal histone tails are shown and may be epigenetically modified. **b** Chromatin consists of multiple nucleosome units strung together by double-stranded DNA. **c** Schematic representation of known modifications of the first 30 amino acids at the amino-terminal tail of H3. The lysine residues at positions 4 and 9 are indicated in red. *M* methylation; *A* acetylation

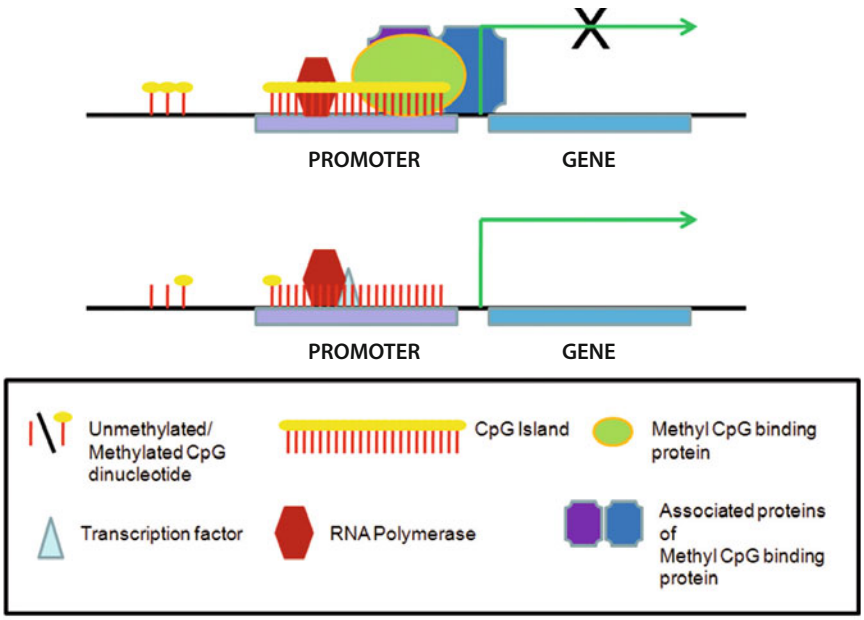

Fig. 5.2 Gene expression is modulated by nearby DNA sequences called promoters, which often include CpG islands. Methylated DNA (*top*) recruits methyl-CpG binding proteins that in turn recruit associated proteins to inhibit transcription. In contrast, unmethylated CpG islands (*bottom*) allow binding of RNA polymerase and transcription factors for initiation of transcription

silencing, while that on the fourth residue (H3K4me3) promotes gene expression.

5.2.2 DNA Methylation

DNA methylation involves the addition of a methyl group on the C5 position of cytosine residues in CpG dinucleotides (cytosine linked to guanine by a phosphate group), in a process mediated by DNA methyltransferases. A genomic region with an enrichment of CpG dinucleotides is called a CpG island. CpG islands are usually found at the promoter regions of many genes.

The methylation status of CpG islands within promoter sequences exerts a regulatory effect by modifying the binding affinity of transcription factors (specific proteins which facilitate DNA recognition by RNA polymerases) to their target sites on DNA, hence modulating transcription levels (Fig. 5.2). CpG islands are not normally methylated, thereby

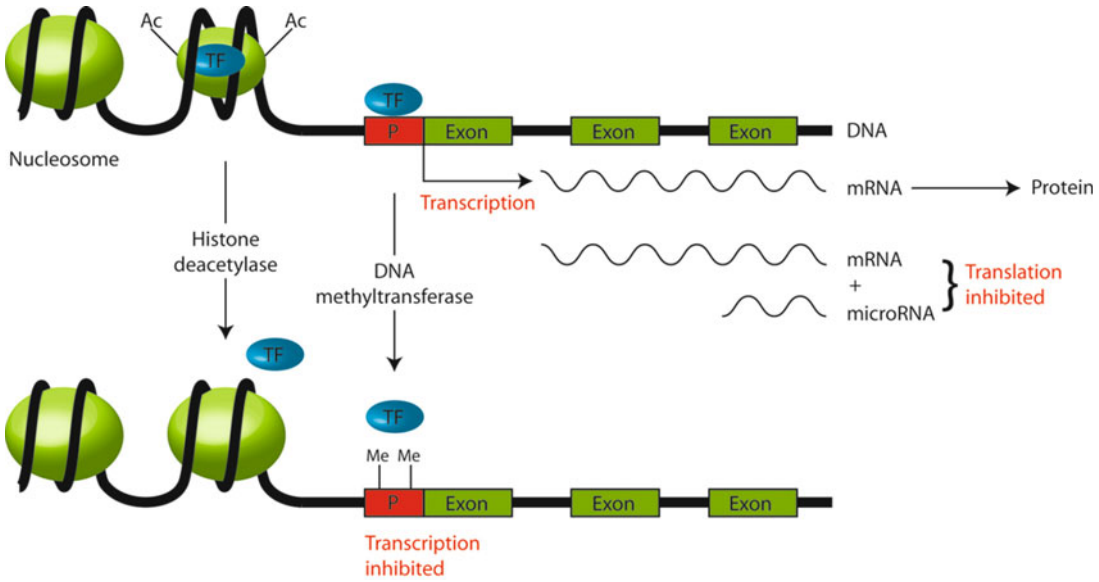

Fig. 5.3 In transcriptionally active chromatin (*top*), acetyl groups (Ac) on specific lysine residues of core histones in the nucleosome decrease their binding to DNA. The resulting "open" chromatin structure, coupled with the unmethylated state of CpG sequences in promoter regions of actively transcribed genes, facilitates access of transcription factors to DNA. Conversely transcriptionally inactive chromatin (*bottom*) is characterized by histone deacetylation and promoter CpG methylation (Me), and hence decreased binding of transcriptional factors. miRNA molecules provide post-transcriptional epigenetic control by binding to complementary sequences in the 3' end of mRNA, thus reducing the rate of protein synthesis. Modified from [7], with permission

allowing transcription to proceed. Methylated DNA however recruits methyl-CpG binding proteins such as MeCP1 and MeCP2, which in turn recruit associated proteins that cause the nucleosome to condense, thereby inhibiting transcription (Figs. 5.2 and 5.3). DNA methylation is therefore commonly associated with gene silencing, although there is increasing evidence that this may be site-dependent. It is also a key factor in other epigenetic events such as X-chromosome inactivation, tissue-specific gene expression during cell differentiation, and genomic imprinting (see later).

5.2.3 Regulatory RNAs

The regulatory role of RNA began to emerge upon the discovery of RNAs that are not translated into proteins. These so-called non-coding RNAs (ncRNAs) are processed by enzymes such as Dicer and Drosher into shorter fragments, yielding different classes such as short interfering RNA (siRNA) and micro-RNA (miRNA), which are approximately 22 nucleotides long, or small nucleolar RNAs (snoRNA) which are ~60–300 nucleotides long [3].

Mature miRNA molecules interact by base-pairing with target messenger RNA (mRNA), modulating translation or direct degradation of the double-stranded RNA molecule. Similarly, siRNAs are perfect matches for their target RNAs, which are then destined for degradation [4]. Gene expression can therefore be regulated at the post-transcriptional level (Fig. 5.3).

SnoRNAs act as guides of specific protein complexes that perform specific nucleotide modifications by base-pairing with their targets near modification sites. SnoRNAs have been implicated in physiological functions that regulate feeding and growth, and in pathological conditions such as the Prader-Willi and Angelman syndromes (see later) and cancer [3].

5.2.4 Genomic Imprinting

Some mammalian genes are expressed from only the maternal or paternal allele – a phenomenon known as genomic imprinting. Many imprinted genes play major roles in fetal growth and nutrition as they are required for the formation of a functional placenta. The first genes shown to be imprinted were the fetal growth factor *IGF2* and its receptor *IGF2R*. More than 100 imprinted genes have been identified to date in mice and humans, with a majority of them located in clusters throughout the genome. These clustered genes usually contain a few protein coding genes and at least one ncRNA gene. Each gene cluster is regulated by a cis-acting element called the Imprinting Control Region (ICR). Between them, the ICRs of the chromosome pair acquire different levels of

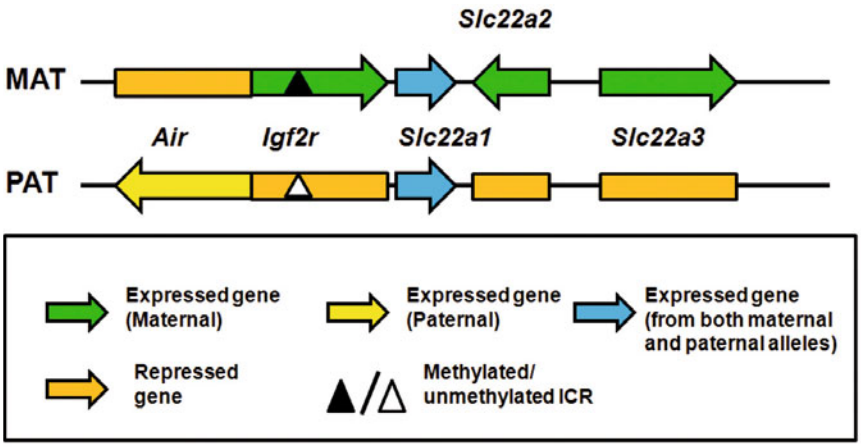

Fig. 5.4 The *Igf2r/Air* imprinted region. *Igf2r*, *Slc22a2* and *Slc22a3* are maternally expressed protein coding genes. *Slc22a1* is a biallelically expressed gene (expressed from both maternal and paternal alleles). *Air* is paternally expressed and produces an ncRNA. The ICR contains the *Air* promoter and is methylated on the maternal allele. When the *Air* transcript is truncated, biallelic expression of *Igf2r*, *Slc22a2* and *Slc22a3* is acquired, indicating a role for the *Air* ncRNA in the repression of the paternal alleles

methylation; this determines which allele is silent or active, thereby enabling imprinting control of all the genes within the cluster [5]. An example of the imprinted mouse gene *Igf2r* and how it is regulated is shown in Fig. 5.4.

A consequence of genomic imprinting is that viable embryos must receive two haploid genome complements from each parent. Parent-specific imprinting marks are established during gametogenesis. Deletions, duplication, mutations or alterations of imprinting of the only active allele, or loss of imprinting of the inactive allele, leads to an imbalance in the dosage of the gene product. The loss or gain of function of imprinted genes may have phenotypic consequences.

5.3 Developmental Plasticity, Epigenetics and Disease Risk

The influence of fetal environment on later-life susceptibility to chronic, non-communicable disease has been demonstrated by a multitude of epidemiological and experimental studies. In humans, in utero exposure to adverse conditions such as maternal under-nutrition has been associated with hypertension, ischemic heart disease, glucose intolerance, insulin resistance, type 2 diabetes mellitus, obesity and reproductive disorders in adulthood [6]. In rodent models used to simulate human pathology, manipulation of maternal nutrition (e.g., food intake or protein restriction), stress (e.g., via glucocorticoid administration) or physical condition (e.g., ligation of uterine arteries to reduce uteroplacental blood flow, thus inducing intrauterine growth restriction) can induce slowed fetal growth and permanent changes in cardiovascular and metabolic function in the offspring [7]. The fetus' ability to respond to challenging environmental cues and adjust its developmental trajectory to match its environment reflects the processes of developmental plasticity. Although these responses are adaptive and aimed at promoting fitness later in life, a mismatch between the eventual phenotypic outcome and the later environment may manifest as chronic non-communicable disease, and this is the basis for the "developmental origins of health and disease" paradigm [6]. Indeed, there is growing evidence that developmental plasticity can account, at least in part, for a broad array of diseases.

The mechanistic basis of the effect of early environmental cues on subsequent susceptibility to metabolic disease has been investigated with animal studies. For example, macaque fetuses whose mothers received a high-fat diet demonstrated impaired lipid metabolism in association with a raft of epigenetic changes such as increased histone acetylation. In rats, a low-protein maternal diet resulted in hypertension and endothelial dysfunction in offspring, with concomitant changes detected at the epigenetic and gene expression levels; these included gene promoter hypomethylation, and accordingly protein overexpression, of hepatic glucocorticoid receptor (GR) and peroxisome proliferator-activated receptor-α (PPAR-α). A recent study in children found that a mother's carbohydrate intake during early pregnancy was associated with methylation of the *RXRA* gene promoter, which in turn could account for more than 20% of variance in adiposity at age 6 or 9 years [8].

The epigenetic mechanisms underlying several imprinting disorders are well established. Human chromosome 15q11-13 contains an imprinted cluster called the Prader-Willi/Angelman syndrome (PWS/AS) region. Incorrect establishment of imprinting in this region results in two distinct neurological disorders, Prader-Willi syndrome (PWS) and Angelman syndrome (AS). PWS is characterized by multiple symptoms including hypotonia, hyperphagia, obesity and short stature. Psychomotor development is mildly affected and behavioral problems are evident. Patients with AS show a completely different phenotype characterized by severe mental retardation, absent speech, autistic-like behavior, severe epilepsy and postnatal microcephaly [8]. PWS is caused by the loss of the normal paternal contribution of gene expression, whereas AS is caused by the loss of the normal maternal contribution.

Other imprinting disorders include Silver-Russell syndrome and Beckwith-Wiedemann syndrome, which arise

from imprinting defects in human chromosome imprinted region 11p15.

Rett syndrome is a devastating X-linked neurological disease that affects females. Girls with this condition appear to develop normally, but show signs of deteriorating motor and cognitive function such as repetitive teeth-grinding, hand-wringing and autistic-like behaviors sometime between the age of 6 and 18 months. This eventually culminates in severe intellectual disability. Rett syndrome is associated with the failure of mutated *MeCP2* to regulate transcription of a specific gene, *DLX5*, one allele of which is normally imprinted. Absence of *MeCP2* leads to increased production of DLX5 protein through the loss of silent chromatin and the activation of additional neighboring chromatin [10], ultimately affecting brain development. Rett syndrome is illustrative of how different epigenetic mechanisms – in this case loss of imprinting and chromatin remodeling – can act in concert to cause various perturbations in gene expression.

5.4 Reversibility of Developmentally-Induced Epigenetic Changes

The "plastic" responses demonstrated by the developing fetus to cope with an adverse prenatal environment have generally been considered to be irreversible. However, our work with animal models has demonstrated the possibility of reversing phenotypic fates induced in utero or in early development. For example, offspring of undernourished rats develop obesity, hyperinsulinemia and hyperleptinemia in adulthood, especially in the presence of a high-fat diet. Intervention by way of neonatal leptin administration to primed rat pups normalized weight gain, insulin levels and leptin concentrations. The corrective effects on phenotype were accompanied by normalized promoter methylation and expression of *Ppar-alpha* [7, 11], reflecting an epigenetic basis for the physiological mechanisms that lead to either phenotype. Other studies have suggested that dietary intervention using n-3 fatty acids may reverse hyperleptinemia and hypertension.

In the rat, variations in postnatal maternal care stably alter the development of behavioral and endocrine responses to stress in the offspring. High levels of maternal care over the first week of postnatal life are associated with reduced fearfulness and a dampened hypothalamic-pituitary-adrenal axis response when exposed to stress. Interestingly, pharmacologic administration in adulthood may alter these behavioral responses by changing the epigenetic status of relevant genes, in particular the hippocampal *GR*. The administration of the HDAC inhibitor trichostatin A in stress-challenged offspring that had received lower maternal care induces behavior more similar to that of offspring exposed to high levels of maternal care. The methyl donor L-methionine induces the opposite effect, with adult offspring exposed to high maternal care exhibiting a potentiated behavioral response to stress. The effects of these interventions have been linked to methylation and expression levels of hippocampal GR [12].

At present, the prevention or reversibility of developmentally induced epigenetic changes has only been investigated with animal models. Nevertheless, the contribution of epigenetic mechanisms to the elucidation of developmental pathways to human disease is now well recognized. It appears increasingly likely that specific epigenetic marks can serve as biomarkers of later life disease risk. The clinical implications of such prognostics are clear – by identifying individuals with increased susceptibility to chronic disease in later life, appropriate nutritional, pharmacological or educational interventions could be then employed as preventative measures. A particular focus on the prenatal and early postnatal periods appears to be prudent.

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6

Congenital Malformations and Syndromes: Early Diagnosis and Prognosis

Giovanni Corsello and Mario Giuffrè

6.1 Introduction

Congenital malformations are defects of the morphogenesis of organs or body regions identified during intrauterine development or at birth. They may be isolated and single, or multiple. Their global birth prevalence is about 2–3%. Congenital defects may be caused by genetic and/or environmental factors, acting singly or in combination. Diagnostic and therapeutic tools have allowed better identification of congenital malformations and have reduced long-term morbidity and mortality in affected patients. Because of increased life expectancy, congenital malformations today represent a major issue in health care because of the resources needed for multidisciplinary care.

6.2 Classifications

Based on clinical criteria, *major malformations* are defined as defects causing functional impairment and therefore needing medical or surgical treatment. Defects that do not produce functional impairment and do not require medical intervention are termed *minor malformations* if their prevalence at birth is less than 4% and *phenotypic variants* when the birth prevalence is higher. Major and/or minor congenital malformations are frequently associated; apparently isolated defects may be associated with malformations that are not clinically evident at birth.

On the basis of etiological criteria, it is possible to distinguish primary malformations, secondary malformations (disruptions) and deformations (Table 6.1). *Primary malformations* are morphogenic defects arising from an intrinsic error of development with a genetic origin. *Disruptions* occur when an environmental factor interferes with an otherwise normal developmental process, causing global impairment or specific damage affecting a single developmental region. The causes of secondary malformations may be biological,

Table 6.1 Etiologic classification of congenital malformations

Primary (genetic)	Secondary (environmental)
Chromosomal abnormalities • Numeric – polyploidy – polysomy – monosomy • Structural – deletions – duplications – insertions – translocations Monogenic • Point mutations – nonsense – mis-sense – frameshift • Dynamic mutations – triplet amplification • Epigenetic regulation – imprinting defects – uniparental disomy Polygenic – oligohydramnios – uterine malformations	Biologic agents • Viruses – cytomegalovirus – rubella – herpes viruses • Bacteria – *Treponema pallidum* • Parasites – *Toxoplasma gondii* Chemical agents • Drugs – antiblastics – anticonvulsants – antibiotics • Abuse substances – alcohol – smoke – cocaine – opiates • Metabolic conditions – hyperglycemia, hyperinsulinemia – hyperphenylalaninemia – hyperandrogenism • Physical agents – ionizing radiation – electromagnetic radiation • Vascular disruptions – subclavian artery vascular disruption – twin-twin disruption sequence • Mechanical causes (deformations) – amniotic bands – twinning – uterine tumors

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

chemical, metabolic, or physical. *Deformations* arise from extrinsic mechanical compression of one or more regions of the body during fetal development. The most common causes of deformations are amniotic bands, twinning, uterine malformations and masses.

Patients with multiple malformations can be classified as having syndromes, sequences, associations, or dysplasias. *Syndromes* are conditions where all the structural defects arise from a single etiological factor, which may be genetic or environmental. *Sequences* are characterized by a cascade of dysmorphic processes, linked by a cause/effect relationship with a single initiating event. Thus, a primary defect may induce several secondary defects, which are chronologically and pathogenetically related. *Associations* are sporadic events with different defects being present more frequently than would be expected if they were random events, without evidence of any etiological or pathogenetic relationship. In recent years, some associations have been recognized as disorders of blastogenesis, which are caused by genetic or environmental factors that interfere with early development when the embryo is a single developmental field, and producing defects in apparently unrelated organs and body regions. Blastogenesis is the first 4 weeks after conception and is characterized by processes that determine the midline and body axes, symmetry and lateralization, neurulation and somite formation. Some associations are identified by an acronym of the defects, such as VACTERLS (Vertebral defects, Anorectal atresia, Cardiac anomalies, Tracheoesophageal fistula, Esophageal atresia, Renal anomalies, Limb defects, Single umbilical artery) and CHARGE (Coloboma, Heart disease, Atresia of choanae, Retarded mental development, Genital hypoplasia, Ear anomalies). *Dysplasias* are structural defects involving specific tissues where a single gene mutation may determine the abolition or reduction of protein synthesis as well as the production of defective proteins.

6.3 Clinical Considerations

The birth of a baby with congenital malformations is the starting point of a clinical process that is aimed at making a precise diagnosis [1, 2]. This leads to appropriate clinical planning and definition of prognosis and counselling of the parents. Different causal pathways may lead to a similar phenotype [3, 4] and the diagnostic process may be long and difficult, requiring follow-up to establish the natural history of the disorder. The diagnostic process includes an accurate history, description of the phenotype, with appropriate imaging and laboratory tests. Improvements in informatics have led to the development of computerized systems to improve diagnostic accuracy. The history should include consideration of the whole family (with the definition of a genealogical tree and the identification of risk factors (consanguinity, multiple abortions, stillbirths, advanced maternal and/or paternal age), the preconceptional period and environmental factors (infections, maternal metabolic diseases, diabetes mellitus, drugs, alcohol) and the pregnancy. Analysis of the phenotype is aimed at the identification and description (with photographic documentation) of structural defects (isolated and multiple, major and minor) and should include a description of clinical associations with genetic syndromes, such as neuropsychomotor retardation, growth restriction, disorders of sexual differentiation or pubertal development.

6.4 Genetic Counselling and Prenatal Diagnosis

Genetic counselling is defined as the non-directive process of communicating with and giving information to a family (usually the parents) to enable the making of decisions relating to patients with genetic diseases that are considered, responsible and rational. It is important that genetic counsellors have: (a) a confirmed diagnosis for the index patient, (b) an up-to-date knowledge of the natural history of the disease, (c) an understanding of its prognostic and therapeutic implications, (d) knowledge of its pattern of inheritance, and (e) an awareness of the possibilities for early (prenatal) diagnosis. The main factors that determine a need for genetic counselling are the presence of an index case with a congenital malformation or of genetic disease in the family as well as the presence of parental risk factors (consanguinity, advanced maternal age, recurrent abortions, mutation carrier). If the disease is multifactorial (due to a combination of genetic and environmental factors), a recurrence risk may be given only on the basis of an empirical approach that takes account of the prevalence in a certain population or at a certain maternal age, number of affected subjects and consanguinity within the family. In the case of monogenic diseases with Mendelian inheritance (autosomal dominant, autosomal recessive, X-linked), the relative recurrence risk must be explained to the family, who must also be informed about available opportunities for early diagnosis (preconceptional, prenatal, neonatal).

The recent identification of complex genetic mechanisms (genomic imprinting, uniparental disomy, triplet amplification) has increased the number of diseases in which is possible to give helpful genetic counseling.

6.5 Associations

Associations are usually sporadic conditions with a low recurrence risk. Environmental factors (alcohol, drugs, maternal diabetes) as well as chromosomal and single gene disorders may interfere with the blastogenic processes. A genetic background may also increase an individual's susceptibility to environmental factors

6.5.1 VACTERLS Association

Characterised by the manifestation of some or all of the defects summarized by the acronym: Vertebral defects, Anorectal atresia, Cardiac anomalies, Tracheoesophageal fistula, Esophageal atresia, Renal anomalies, Limb defects, Single umbilical artery. Tracheoesophageal and anorectal defects require early surgery during the neonatal period. The most frequent heart defects affect the ventricular septum. Limb defects include radial ray abnormalities (radial hypoplasia, thumb aplasia or hypoplasia as well as its duplication), polydactyly and syndactyly. Other features may be prenatal and postnatal growth restriction, ear and external genitalia abnormalities. VACTERLS association is often sporadic with a low recurrence risk. It is more frequent in the offspring of diabetic mothers. In some patients with associated obstructive hydrocephalus, gene mutations with Mendelian inheritance have been documented. Most patients have normal cognition, although they may present as failure to thrive and with neuromotor disabilities.

6.5.2 Infants of Diabetic Mothers

Congenital malformations in the offspring of diabetic mothers are clinically heterogeneous. Their frequency is 2–4 fold higher than in the general population. The relative risk for malformations is much higher in newborns of women with type I insulin-dependent diabetes mellitus and is inversely correlated with the effectiveness of maternal glycemic control, particularly during the periconceptional period. Several factors are involved in the pathogenesis of such defects: hyperglycemia, hyperglycosylation of proteins involved in differentiation, chronic hypoxia, polycythemia/hyperviscosity, and lactic acidosis. All these factors may interact and interfere with blastogenesis, inducing abnormalities of the midline structures and symmetric organs. All types of malformations (skeletal, cardiac, renal, gastrointestinal, CNS) are more common in the infants of diabetic mothers. Some are specific, such as caudal dysgenesis, characterized by defects of vertebral, urogenital and intestinal structures arising from the caudal mesoderm, with a wide spectrum of expression including sirenomelia, in which there is also a complex vascular defect. The fetus may be macrosomic because of fetal hyperinsulinemia, although a placental microangiopathy may also cause intrauterine growth restriction.

6.6 Sequences

Malformation sequences may be caused by genetic as well as environmental factors. Several organs and systems may be involved in malformation sequences (Table 6.2).

6.6.1 Holoprosencephalic Sequence

Holoprosencephalic sequence presents at birth with a wide spectrum of defects of the encephalon and the median craniofacial areas due to a developmental defect of the median subdivision of the prosencephalic vesicle and surrounding mesoderm. It is characterized by a wide range of etiological factors, being found in patients with chromosomal aberrations, monogenic mutations and environmental disruptions (gestational diabetes, cytomegalovirus).

Three anatomical variants (alobar, semilobar and lobar) and four clinical variants (cyclopia, ethmocephaly, cebocephaly and premaxillar agenesis) have been described. Clinical evaluation must include CNS imaging to define the full phenotype. The severity of CNS defects is the main reason for the high and early lethality of the condition. If the phenotype is only partially expressed, longer survival is possible,

Table 6.2 Main malformation sequences

Name	Developmental field and organs involved
Holoprosencephaly	Precordial mesoderm, prosencephalic vesicle, rinencephalon, orbits, nose, premaxilla
Septo-optic dysplasia	Optic chiasm, hypophysis
Pierre Robin	Jaw bone, oro-pharyngeal region
DiGeorge	4th brachial arch, 2nd and 3rd brachial pouches
Poland	Pectoral muscle, superior limb
Klippel-Feil	Spine
Potter	Kidneys, urinary tract, lungs, limbs, facies
Prune belly	Urinary tract, abdominal wall
Bladder-cloacal extrophy	Peri-umbilical mesoderm
Rokitanski	Muller ducts
Sirenomelia	Caudal mesoderm
Caudal regression	Caudal mesoderm
Premature rupture of amnion	Median axis, limb deformations, facial clefts
Fetal akinesia	Multiple body regions
Twin-twin disruption sequence	Multiple body regions

frequently associated with relevant neurologic problems. The parents and relatives of patients must be investigated for minor signs of the sequence (hypotelorism, single median incisor) in order to recognize a possible autosomal dominant inheritance. Most cases are multifactorial and the recurrence risk for families with a sporadic case is estimated at around 6%.

6.6.2 Pierre Robin Sequence

Pierre Robin sequence is a developmental defect of the mandible and surrounding oro-pharyngeal region. It is characterized by microretrognathia, cleft palate and functional disturbances (swallowing deficit, respiratory distress). A deficiency of mesoderm induction may cause a primary cleft palate (V-shape). Alternatively, the cleft palate may be secondary to jaw arch hypoplasia, which leads to the position of the tongue being fixed between the palatine processes, causing a defect of fusion of the secondary palate in the midline (U-shape). Respiratory obstruction is caused by the tongue falling backwards and by a primitive pharyngeal stenosis (secondary to a migration deficit of neural crest cells). The sequence may present with a wide phenotypical spectrum, either in isolation or as part of a more complex syndrome (del 18q, Stickler syndrome). All these variables influence the prognosis. Newborns may require prolonged respiratory and/or nutritional support, long-term hospitalization and follow-up. In the most severely affected cases, surgery to the jaw distraction may improve the outcome.

6.6.3 Potter Sequence

The Potter sequence is caused by absent or severely reduced fetal urine output from the first trimester. It may be secondary to bilateral renal agenesis (a differentiation defect of the metanephric blastema) or to other renal and urinary tract malformations. The cascade mechanism starts with diminished urine production by the fetus; this leads to a reduced volume of amniotic fluid (anhydramnios or oligohydramnios), which give rise to pulmonary hypoplasia, leading to respiratory distress at birth, facial dysmorphism (prominent nose and flat profile), diminished fetal movements with multiple postural deformations particularly of the lower limbs. When the phenotype is fully expressed, the severe renal and pulmonary damage is responsible for the high perinatal mortality. Prenatal diagnosis by ultrasound reveals the kidney defect, oligohydramnios and other associated malformations. The sequence is often sporadic, with etiological heterogeneity and a recurrence risk of about 3%. It may occasionally be associated with other defects in a more complex syndrome (Meckel-Gruber syndrome: occipital encephalocele, renal cystic disease, polydactyly and an autosomal recessive inheritance).

6.6.4 Prune Belly Sequence

This sequence was named because of the characteristic appearance of the abdomen (wrinkled skin, also referred to as "flabby abdomen") in the affected newborns. The sequence may be related to various defects of the genitourinary tract, involving the proximal urethra (urethral agenesis, cloacal persistence, urethral stenosis, posterior urethral valves in males) [5]. Urethral obstruction is responsible for oligohydramnios (and possible secondary Potter sequence) and accumulation of urine in the proximal renal tract, leading to parenchymal damage (bladder dilatation, bilateral ureteric dilatation and hydronephrosis). Bladder hypertrophy and dilatation may interfere with development of the abdominal wall muscles, diaphragm and testicular migration in the scrotum in males (cryptorchidism). Abdominal wall muscle hypoplasia is responsible for the prune belly appearance because of visible intestinal loops through the thin abdominal wall. Diaphragmatic defects and oligohydramnios cause lung hypoplasia and severe respiratory distress at birth. Although early prenatal diagnosis by ultrasonography is possible, the differential diagnosis between isolated renal cystic conditions and obstructive uropathies may be difficult. Prenatal bladder catheterisation allows urine to flow into the amniotic cavity and must be followed by surgical correction after birth.

6.7 Syndromes

6.7.1 Chromosomal Abnormalities

The overall incidence of chromosomal anomalies is estimated at about 1:170 live births. Their prevalence at conception is much higher, giving rise to a spontaneous abortion or fetal death because of developmental impairment. About 50% of spontaneous abortions have an abnormal chromosomal structure. Chromosomal aberrations may affect *autosomes* and/or *sex chromosomes* and may involve their number or structure. *Numeric aberrations* have a prezygotic origin (meiotic non-disjunction, frequently related to advanced maternal age). They may also arise from a postzygotic error when they are present only in a variable proportion of cells (mosaics). *Structural aberrations* may occur de novo from a meiotic rearrangement or may be inherited from one parent, who carries a balanced non-symptomatic chromosomal translocation.

6.7.1.1 Down Syndrome (Trisomy 21)

Down syndrome is the most frequent chromosomal aberration at birth (about 1:700). It is determined by a trisomy of chromosome 21. In most (95%) cases, trisomy 21 is secondary to a maternal meiotic non-disjunction of homologous chromosomes

21, more rarely there may be a Robertsonian translocation or a post-zygotic mitotic non-disjunction (mosaic with milder phenotype). The incidence is related to maternal age at conception (1/1500 at 20 years and 1/28 at 45 years). Overall recurrence risk is low (about 1%), although it significantly increases when one of the parents carries a balanced translocation. The phenotype at birth is characteristic: main facial features are Brushfield spots (grey spots in the median zone of the iris), upslanting palpebral fissures, epicanthal folds, small nose, small mouth with prominent tongue, flat facial profile, brachycephaly with a flat occipital bone, small low-set ears, short neck with redundant skin folds. Newborn babies are hypotonic with lax joints. A single palmar crease and clinodactyly of the little finger are frequent. Organ involvement includes congenital heart defects (atrioventricular canal, ventricular septal defects, tetralogy of Fallot), duodenal atresia or stenosis, Hirschsprung's disease, hypothyroidism and urinary tract malformations. Long-term follow-up is required because of psychomotor and mental retardation, growth retardation, occurrence of autoimmune diseases, immunodeficiencies and leukemia. Survival rates and the quality of life have improved significantly with educational and screening programs and the development of multidisciplinary follow-up.

6.7.1.2 Edwards Syndrome (Trisomy 18)

Edwards syndrome is determined by trisomy of chromosome 18, sometimes as a mosaic or in association with other chromosomal abnormalities. Its birth prevalence is about 1:8000 because most affected fetuses abort spontaneously. Newborns show severe prenatal growth restriction, dolicocephaly with prominent occiput and low-set dysplastic external ears, jaw hypoplasia, flexed hands with the index finger overlapping the middle finger, single palmar crease, and talipes with rocker-bottom feet. There are frequently associated malformations (heart, renal, intestinal, CNS), which are responsible for the very grave prognosis and high neonatal mortality.

6.7.1.3 Patau Syndrome (Trisomy 13)

Determined by trisomy of chromosome 13, sometimes with chromosomal translocation, or rarely as mosaic. Its birth prevalence is about 1/10,000. Newborns show prenatal growth restriction, a small trigonocephalic skull, areas of aplasia cutis on the scalp, cleft lip and palate, microphthalmia, variable hypotelorism up to cyclopia (i.e., expression of an associated holoprosencephalic sequence), postaxial polydactyly and/or syndactyly, forced flexion of the fingers, single palmar crease, plantar convexity, and cryptorchidism. Other organs are frequently involved (heart, kidneys). Patients with fully expressed phenotype usually die during the first month of life, those with milder signs (mosaics) may survive with severe developmental deficits.

6.7.1.4 Wolf-Hirschhorn Syndrome (4p-)

This is a rare condition determined by deletion of the distal part of the short arm of chromosome 4. Newborns present with prenatal growth restriction, hypotonia, severe microcephaly with brachycephaly, prominent nose, downturned corners of the mouth, arched palate, jaw hypoplasia, hypertelorism, downslanting palpebral fissures, iris coloboma, large low-set external ears. Heart, renal and skeletal defects are frequent. The degree of extension of the chromosomal deletion influences the severity of phenotype and neonatal mortality rate. Surviving patients show severe postnatal growth retardation and psychomotor developmental delay.

6.7.1.5 Cri-du-chat Syndrome (5p-)

Cri-du-chat syndrome is due to a variable deletion of the short arm of chromosome 5. Named because of the characteristic high-pitched cat-like cry of affected newborns caused by hypoplasia of laryngeal cartilages, which disappears after the first months of life. Other phenotypical features are microcephaly, round face, hypertelorism, micrognathia, epicanthal folds, low-set ears. At birth there is generalized hypotonia. Later there is limb hypertonia and severe psychomotor and mental retardation.

6.7.1.6 Mosaic 8 Chromosome Trisomy

The full trisomy of chromosome 8 is extremely rare in humans. It is more common as a mosaic. The phenotype of mosaic trisomy 8 includes scaphocephaly, ankylosed large joints, clubfoot, absent or hypoplastic patellae, arachnodactyly and brachydactyly. Deep grooves in the palms and soles (Fig. 6.1)

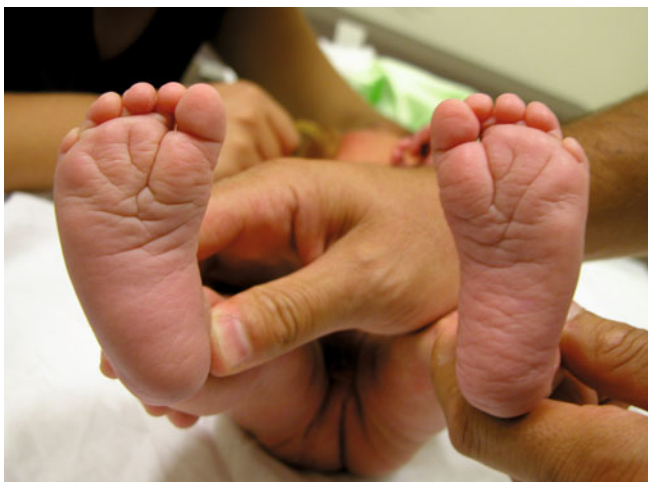

Fig. 6.1 Deep plantar grooves in a newborn with mosaic 8 chromosome trisomy

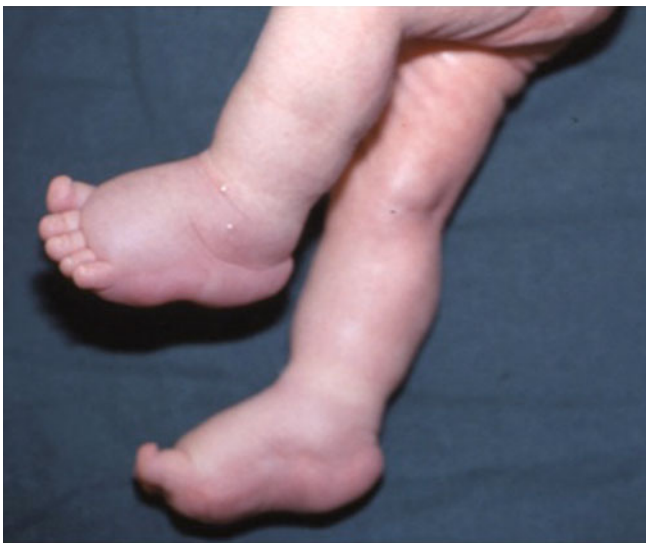

Fig. 6.2 Foot lymphedema in a baby with Turner syndrome

are virtually diagnostic in infancy but become less prominent with age. The face is characterized by a prominent pouting lower lip and small jaw. Mental retardation may be present but is often mild and may remain undetected.

6.7.1.7 Turner Syndrome

Turner syndrome is the most frequent aneuploidy of sex chromosomes with a birth prevalence of 1/2500. It is determined by a monosomy of chromosome X, which may be complete (50%) or partial (20%); it frequently presents as a mosaic with a milder phenotype (30%). During the neonatal period, the diagnosis is suspected because of lymphedema of the hands and feet (Fig. 6.2), nail dysplasia, neck pterygium, a large mouth with downturned corners, dysplastic external ears and left heart output defects (aortic coarctation, left heart hypoplasia). In addition, there may be a prenatal history of cystic hygroma. The clinical phenotype changes with age when other features become evident: short stature, short neck, low posterior hairline, restricted thorax, cubitus valgus, shortness of the fourth metacarpal bone, primary amenorrhea, absence of secondary sexual signs, an endocrine profile of gonadal dysgenesis. Life expectancy is not reduced, but long-term follow-up is required and appropriate hormonal therapy (growth hormone in the first decade and oestrogen-pregesterone after puberty must be given in order to improve height and induce the menstrual cycle.

6.7.1.8 CATCH 22 Syndrome

CATCH 22 is an acronym of the main clinical features (Cardiac abnormality, Abnormal face, Thymic hypoplasia, Cleft palate, Hypoparathyroidism, chromosome 22 microdeletion). This syndrome combines some clinical conditions which were previously described separately (Velo-Cardio-Facial syndrome, Di George sequence). It is due to a deletion of the chromosomal region 22q11.2. Clinical expression is variable and FISH analysis is required to confirm the diagnosis and to define the extent of deletion and the genes involved (contiguous gene syndrome) [6]. Heart defects are conotruncal (aortic arch interruption, common arterial trunk, tetralogy of Fallot). When the brachial structures are involved, there is dysfunction of the immune system (T-cell deficiency) and parathyroidal abnormalities with low serum calcium levels. During the neonatal period, the full expression of the phenotype results in hypocalcemia and craniofacial dysmorphism (micrognathia, cleft palate, anteverted nares, low-set external ears) with a conotruncal heart defect and absent thymic shadow at chest X-ray delineating the phenotype with full expressivity.

6.7.2 Monogenic Disorders

Monogenic disorders are single gene mutations with a Mendelian mode of inheritance. The genotype-phenotype correlation remains undefined for many conditions. Each syndrome may be due to different mutations in the same gene or in different genes (genetic heterogeneity). The same mutation may determine different phenotypes (phenotypical variability) in the same family and appears to depend on interference by other genetic and/or environmental factors. In addition, epigenetic factors (e.g., DNA methylation) may act during the differentiation processes to modify gene expression and may depend on the parental origin of the gene (genomic imprinting).

6.7.2.1 Cornelia de Lange Syndrome

Affects 1/10,000 newborns and is usually sporadic due to de novo mutations (gene locus at 5p13.1 encoding for the NIPBL protein) [7]. Newborns show a typical facial appearance (microbrachycephaly, low anterior and posterior hairline, synophrys, small nose with a depressed nasal bridge, anteverted nares, long philtrum, "carp" mouth, maxillary prognathism, low-set ears) (Fig. 6.3), with intrauterine and postnatal growth retardation, hypertrichosis and upper limb anomalies (small limbs, reduction defects including phocomelia, limited elbow extension, single palmar crease, oligosyndactyly).

Urogenital, heart and intestinal malformations may also be present. All patients have psychomotor and growth retardation. Infections, feeding difficulties and neurologic disturbances (seizures, motor and speech retardation) require long-term multidisciplinary follow-up and family support.

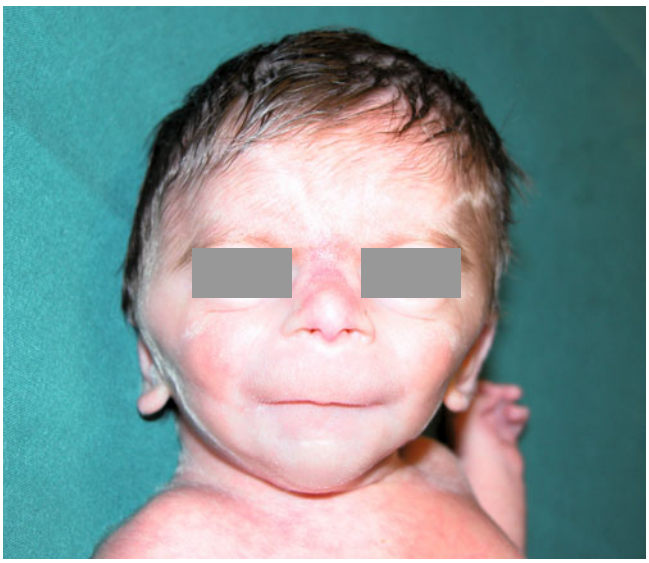

Fig. 6.3 Typical facial appearance of a newborn baby with Cornelia de Lange syndrome, showing synophrys, a depressed nasal bridge, anteverted nares, long philtrum, carp mouth

6.7.2.2 Rubinstein-Taybi Syndrome

A rare sporadic syndrome characterized by mental retardation, facial abnormalities, broad thumbs and toes. There is genetic heterogeneity and about 25% of patients present with mutations or microdeletions in the gene encoding the transcriptional coactivator CREB-binding protein (16p13.3) [8]. Familial cases with autosomal dominant inheritance have been described. The main craniofacial features (Fig. 6.4) are microcephaly, frontal bossing, large anterior fontanelle, downslanting palpebral fissures, broad nasal bridge, beaked nose, epicanthal folds, strabismus, maxillary hypoplasia, high arched palate, external ear abnormalities. There is hand and foot involvement (broad distal phalanges of thumbs and halluces with medial deviation, clinodactyly or duplication). There may also be hirsutism and abnormalities of the skeleton (spinal, pelvic), heart (septal defects, patent ductus arteriosus) and urogenital tract (hypospadias, cryptorchidism). Growth retardation, skeletal maturation delay and severe mental retardation are more common with increasing age.

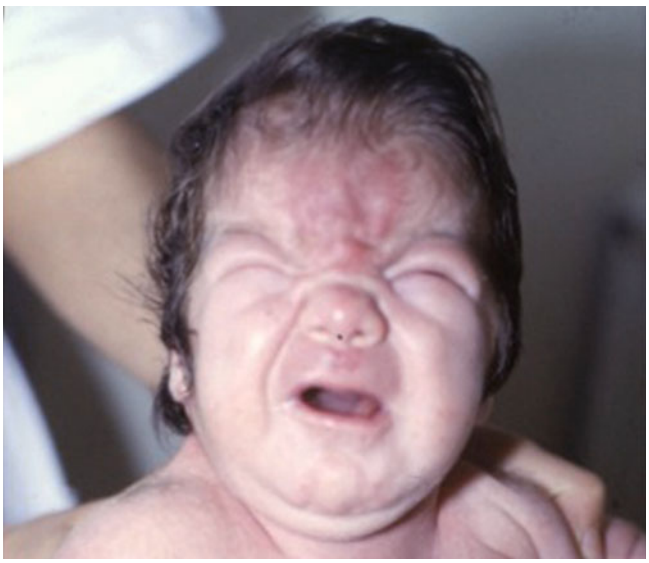

Fig. 6.4 Newborn with Rubinstein-Taybi syndrome showing microcephaly, frontal bossing, downslanting palpebral fissures, broad nasal bridge, beaked nose, epicanthus, maxillary hypoplasia

6.7.2.3 Marfan Syndrome

A congenital defect of connective tissue involving the skeleton, eye and cardiovascular system. It is determined by heterozygous mutations in the *FBN1* gene (15q21.1) encoding for fibrillin 1 protein, which is a component of collagen [9]. It is inherited as an autosomal dominant with variable clinical expression; about 25% of cases are sporadic, due to de novo mutations correlated with advanced paternal age. Newborns show arachnodactyly, long and thin limbs, increased length joint laxity and hypermobility, muscular hypotonia, hernias, pectus carinatum or excavatum. Cardiac abnormalities comprise mitral valve prolapse and aortic defects (aortic root dilatation and aortic aneurysm). Patients with most severe neonatal phenotype (neonatal Marfan syndrome) have high early mortality rates. Skeletal and cardiac problems usually evolve with growth (kyphoscoliosis, progressive aortic dilatation, aortic dissection) and ocular signs develop (ectopia lentis, early glaucoma).

6.7.2.4 Noonan Syndrome

Noonan syndrome is a relatively frequent (1/2000) condition with some phenotypic features similar to those of Turner syndrome (male Turner, pseudo-Turner). It is due to mutations in the *PTPN11* gene (12q24.1), with sporadic as well as familial cases [10]. The inheritance is autosomal dominant and parents must be always investigated for mild clinical signs. Newborns show hypertelorism, upslanting palpebral fissures, low-set posteriorly rotated ears, neck pterygium, a shield chest with deformation of the sternum, and lymphedema (Fig. 6.5). Cardiac involvement is mainly of the pulmonary outflow tract (pulmonary valve dysplasia and stenosis, cuspid thickening and hypomobility). Other possible features are cryptorchidism in males and a bleeding tendency, due to thrombocytopenia and partial deficiency of coagulation factors. Later in infancy, other signs become evident: postnatal growth retardation (with short stature and retarded bone age), triangular face, mild psychomotor and intellectual retardation. Life expectancy depends exclusively on the severity of heart manifestations.

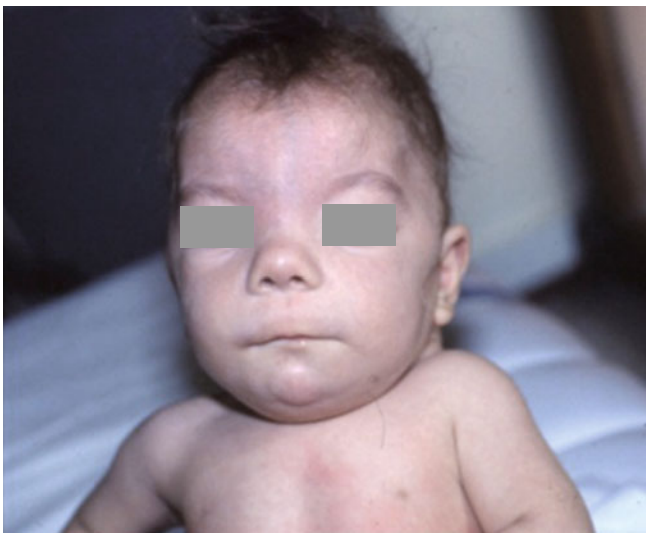

Fig. 6.5 A patient with Noonan syndrome with hypertelorism, upslanting palpebral fissures and shield chest

6.7.2.5 Prader-Willi Syndrome (PWS)

Determined by the failure of expression of genes of paternal origin in the 15q11-q13 region. These genes are normally subjected to maternal imprinting (DNA methylation) which avoids maternal copy transcription and determines a functional gene monosomy (only the paternal copy is expressed). This condition may arise from microdeletions of paternal chromosome 15 (70%), maternal uniparental disomy (25–30%) or imprinting center defects. Methylation testing identifies almost all patients; FISH and microsatellite DNA analysis may reveal microdeletions and maternal UPD. PWS newborns (Fig. 6.6) present with congenital hypotonia, a prenatal history of reduced fetal movements, poverty of facial expression, dolicocephaly with reduced bitemporal diameter, almond-shaped eyes with upslanting palpebral fissures, a small mouth with downturned corners and thin upper lip, small hands and feet and genital hypoplasia with cryptorchidism in males [11]. Hypotonia of the respiratory, orofacial and pharyngoesophageal muscles cause variable degrees of respiratory and feeding difficulties (poor suction and swallowing), which are present from birth and tend to improve after the neonatal period. After the first year of life, the phenotype modifies and is characterized by hyperphagia, obesity, sleep disturbances, short stature, hypogonadotropic hypogonadism, mild to moderate mental retardation, speech delay. Treatment with growth hormone (GH) is possible and good clinical results have been reported.

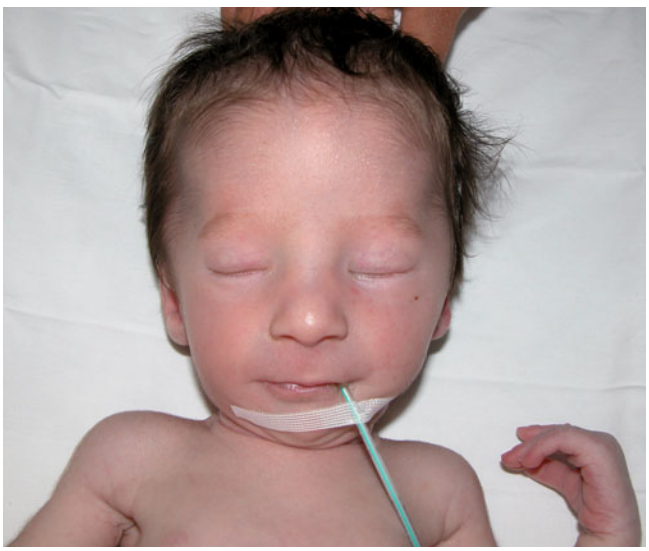

Fig. 6.6 The neonatal phenotype of Prader-Willi syndrome: hypomimic face, reduced bitemporal diameter, almond-shaped eyes, small mouth with downturned corners and thin upper lip

6.7.2.6 Beckwith-Wiedemann Syndrome (BWS)

A sporadic condition determined by an altered balance between cooperating genes in the region 11p15. The genetic mechanism is complex and involves imprinted genes encoding for several important growth factors and receptors. Various genotypic abnormalities have been described in BWS patients, e.g., microduplication of paternal region 11p15, microdeletion of the maternal region, and mutations and paternal uniparental disomy of chromosome 11. The main neonatal clinical features (Fig. 6.7) are exomphalos, macroglossia and gigantism (EMG syndrome). Other features are visceromegaly, adrenocortical cytomegaly, dysplasia of the renal medulla, typical linear indentations of the helix and the auricular lobe of the ear, hypoglycemia during the first days of life, limb hemihypertrophy. Prenatal diagnosis may be because of exomphalos and generalized overgrowth seen on ultrasound scanning. The neonatal diagnosis is based on clinical findings, although careful molecular cytogenetic analysis of the 11p15 region is recommended. Overgrowth and macroglossia tend to become less evident with age, but there is an increased risk of malignant tumors (Wilms' tumor, adrenal carcinoma, hepatoblastoma).

6.7.2.7 Silver-Russell Syndrome

Silver-Russell syndrome is a sporadic condition with genetic heterogeneity. Various genetic loci have been involved [12]. In some patients it is caused by hypomethylation at distal chromosome 11p15 (the opposite of patients with Beckwith-Wiedemann syndrome). In others, it is related to a maternal uniparental disomy of chromosome 7. The neonatal phenotype is characterized by severe intrauterine growth restriction with normal development of the skull (pseudohydrocephalic appearance), poor postnatal growth, craniofacial features (triangular shaped face and broad forehead), body asymmetry and

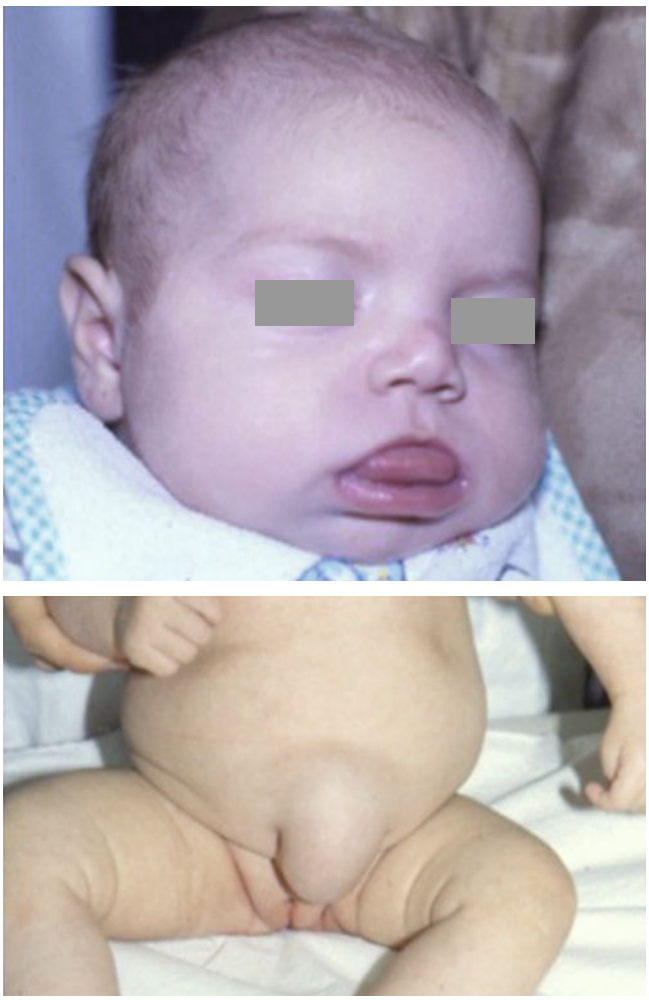

Fig. 6.7 Exomphalos, macroglossia and gigantism

a variety of minor malformations (clinodactyly and syndactyly of the little finger). The phenotypic expression changes during childhood and adolescence, with the facial features and asymmetry usually becoming more subtle with age. GH therapy should be considered to improve final height.

6.7.2.8 Goldenhar Syndrome (GS)

Goldenhar syndrome is a spectrum of malformations involving the eye, ear and vertebrae with heterogeneous etiology and a highly variable phenotype [13]. Its prevalence at birth is about 1/5000. Most cases are sporadic, although some families with autosomal dominant inheritance have been described. GS originates from a vascular disruption of the first and second branchial arches, with consequent malformations of related organs and tissues. Defects are more often unilateral and an increased incidence has been reported in the offspring of diabetic mothers, as have other disorders of blastogenesis. GS newborns show multiple craniofacial anomalies (Fig. 6.8): hemifacial microsomia, ipsilateral deformity of the external ear (preauricular tags, atresia of the external auditory canal, anomalies in size and shape of the external auricle), epibulbar dermoid, coloboma of the upper eyelid. In addition, defects of the cervical vertebrae (hemivertebrae, fusions, segmental agenesis), cleft palate, choanal athresia, heart, kidney and CNS defects may be found. Auditory function must be assessed early to preserve speech and cognitive development.

6.7.2.9 Smith-Lemli-Opitz Syndrome (SLOS)

A rare (1/30,000) autosomal recessive condition, caused by mutations in the gene encoding steroid delta-7-reductase, which maps to the region 11q12-q13 [14]. The enzyme deficiency causes a severe deficit of endogenous cholesterol production and its derivative compounds (sexual steroids, components of the myelin and cellular membranes), starting from intrauterine life. SLOS newborns show intrauterine growth restriction, severe hypotonia, microdolicocephaly, high forehead, palpebral ptosis, anteverted nares and micrognathia. There may be other features, such as agenesis of the corpus callosum, cleft palate, syndactyly of second and third toes, congenital heart defects, liver dysfunction. Male patients usually have hypospadias, micropenis, cryptorchidism and sometimes various degrees of ambiguous genitalia, because of the prenatal androgen deficiency. There are low cholesterol and elevated 7-dehydrocholesterol levels in the blood. The underlying mutation is identified by gene sequence analysis, which allows confirmation of the prenatal diagnosis for at-risk couples. Failure to thrive worsens with age and there is always severe psychomotor delay. Patients may be helped by exogenous cholesterol supplementation, particularly for the biliary acid profile.

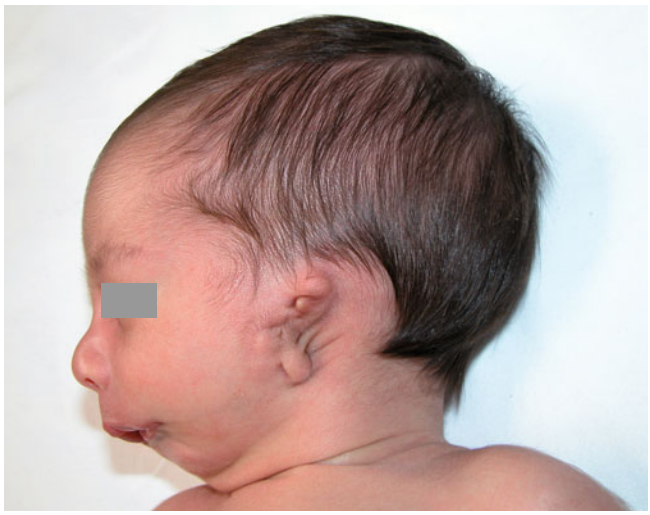

Fig. 6.8 Hemifacial microsomia and atresia of external auricle in a baby with Goldenhar syndrome

6.8 Disruptions

Some congenital malformations can be related to exogenous factors, which act during intrauterine life, inducing abnormalities of developmental and differentiation processes. The most susceptible period is the first trimester of pregnancy in which the identification of developmental fields, organogenesis and differentiation take place. Biological, chemical, metabolic, physical and mechanical agents (Table 6.1) may cause defects of morphogenesis. The etiologic identification is important for effective genetic counselling and reducing the risk of recurrence in an affected family.

6.8.1 Biologic Agents

Most morphogenetic defects determined by biological agents are pathogenetically and clinically characterized. A high IgM level soon after birth is strongly suggestive of a prenatal infection of the fetus, although only 30% of these newborns show clinical consequences. The earlier the timing of intrauterine infection (particularly during the first trimester of gestation), the more severe is the effect of morphogenetic defects due to viral and bacterial agents and there is usually associated intrauterine growth restriction. The most common embryofetopathies due to biologic agents are described in Table 6.3.

6.8.2 Chemical Agents

Any substance introduced to the human organism can be considered as a chemical agent. They may be drugs or foods associated with flawed lifestyle choices (alcohol consumption, cigarette smoking, ingestion of substances of abuse) or produced by maternal metabolism in specific situations. Some drugs and single molecules, which are well tolerated by an adult, may be seriously dangerous for the development of the embryo and fetus. Drug testing in human pregnancy is difficult and all drugs have therefore to be considered potentially harmful, requiring careful risk/benefit evaluation. Substances may cross the placenta and reach the fetus and placental function may reduce or increase their effects. Individual metabolism may influence the clinical effects, dosage and timing of administration. The most frequent disruptions by chemical agents are reported in Table 6.4.

Table 6.3 Main disruptions determined by biologic agents

Biologic agent	Phenotype
Cytomegalovirus	Microcephaly, intracranial calcifications, psychomotor delay, sensorineural hearing loss, chorioretinitis, hepatosplenomegaly, thrombocytopenia, virus presence in secretions and biologic fluids (urine)
Rubella virus	Microcephaly, psychomotor delay, congenital cataract, sensorineural hearing loss, heart defects, hematologic alterations (anemia, thrombocytopenia)
Varicella-zoster virus	Mental retardation, cortical atrophy, seizures, chorioretinitis, skin scars
Treponema pallidum	Palmoplantar pemphigus, exanthema with skin scars, anemia, thrombocytopenia, hepatosplenomegaly, myocarditis, chorioretinitis, muco-hematic rhinitis, skeletal alterations (lacunae, caput quadratum, metaphyseal ossification defects, osteochondrites and secondary pseudoparalysis)
Toxoplasma gondii	Hydrops, hydrocephalus, intracranial calcifications, chorioretinitis, cataract, seizures, hepatosplenomegaly, skin rush

Table 6.4 Main disruptions determined by chemical agents

Chemical agent	Phenotype
Anticonvulsant drugs	Cleft lip and palate, neural tube defects, congenital heart defects – hydantoin (microcephaly, mental retardation, CNS abnormalities, small nose, facial bone hypoplasia, epichantus, hypertelorism, strabismus, cleft lip and palate, micrognathia, short neck, heart defects) – trimethadione (microcephaly, facial bone hypoplasia, palpebral synophrys, epicanthus, external ear dysplasia, urogenital defects, heart defects) – valproic acid (trigonocephaly, reduced bitemporal diameter, facial bone hypoplasia, small nose, cleft lip and palate, urogenital and limb defects)
Alcohol	IUGR, peculiar face (microcephaly, short palpebral fissures, small nose with anteverted nares, hypoplastic nasal philtrum, microretrognathia), neurologic abnormalities (hypotonia, seizures, poor motor coordination, mental retardation)
Cocaine	Prematurity, IUGR, microcephaly, urogenital and skeletal malformations
Heroin	IUGR, low birth weight, congenital malformations
Maternal diabetes	Macrosomia, hypoglycemia, hypocalcemia, ventricular septal hypertrophy, caudal dysgenesis, any kind of congenital malformations (skeletal, cardiac, renal, intestinal, CNS, etc.)
Maternal hyperphenylalaninemia	Defects of cellular proliferation and migration with myelinization delay (IUGR, severe microcephaly, hypotelorism, prominent nose, low-set dysplastic external ears, mental retardation, cleft lip and palate, conotruncal heart defects)

6.8.3 Vascular Disruptions

Any vascular accident during early embryonic and fetal development may determine subsequent morphogenetic defects in the relevant body region.

Disruption of the subclavian artery includes a heterogeneous group of clinically and etiologically different conditions, characterized by alteration of different mesodermal structures supplied by the subclavian artery. Thus, the Poland sequence includes pectoral muscle agenesis and ipsilateral superior limb reduction defects. Kidney and urinary tract defects are frequently associated, expanding the phenotype towards an acro-pectoral-renal developmental field.

Twin-twin disruption sequence (TTDS) may involve various structures such as the brain, brachial arches, limbs, gut, and kidneys. It is caused by the intrauterine developmental impairment and subsequent death of a monozygotic twin. The presence of vascular placental anastomoses between the arterial supplies of twins and abnormal communicating flow allows the passage of thromboemboli to the surviving twin, with reduced or interrupted blood flow causing structural damage. The complex vascular interactions between monozygotic twins may result in other vascular disruptions (e.g., because of an acardic twin) or a twin-twin transfusion sequence, which are particular features of monozygotic twins.

6.9 Dysostoses

A heterogeneous group of birth defects with single or multiple involvement of skeletal segments (with no systemic cartilaginous tissue involvement), due to mutations of the genes involved in bone development. Dysostoses classification is based on phenotypic criteria and the body region most involved. In some instances a genetic classification is now possible. Most syndromal craniosynostoses are determined by mutations in fibroblast growth factor receptor (*FGFR*) genes, with string evidence of genetic heterogeneity (the same condition being determined by different mutations in the same gene or in different *FGFR* genes) and genetic pleiotropism (the same mutation being responsible for different phenotypes). Genotype/phenotype correlation is not possible for all cases and it can be influenced by other genes (epistatic) as well as other interactive cytoplasmic and environmental factors.

6.9.1 Craniofacial Dysostoses

Full fusion of all cranial sutures is normally achieved at about 25 years of age. Craniosynostoses depend on a precocious closure of one or more cranial sutures and may cause a restriction in the size of the cranium. The closure of a suture limits cranial growth at that site and there is increased cranial growth at the other sutures, producing deformation of the skull (sometimes with brain growth restriction, hydrocephalus, and intracranial hypertension). Skull morphology depends on suture involvement (nature, timing, extension and symmetry). The overall incidence of craniosynostoses is estimated to affect about 1/3000 newborns. It may present in isolation or as part of a more complex syndrome.

6.9.1.1 Non-Syndromal Craniosynostoses

Scaphocephaly depends on the premature fusion of the sagittal suture with consequent restriction of growth along the transverse axis and compensatory increased growth along the antero-posterior axis. *Plagiocephaly* depends on the premature fusion of a single coronal suture with consequent ipsilateral growth restriction and flattening of the frontal bone; the involvement of facial structures varies from simple deviation of nasal septum to severe asymmetry of the sphenoid and maxillary bones. *Brachycephaly* is due to premature fusion of both coronal sutures with consequent growth restriction along the antero-posterior axis and compensatory increase in skull height; hypoplasia of the frontal region is often present. *Acrocephaly* depends on the premature fusion of the coronal and sagittal sutures with consequent severe growth restriction along both antero-posterior and transverse axes and compensatory increased development of the frontal region. Intracranial hypertension is frequently present and requires early surgical correction to avoid severe CNS complications. *Trigonocephaly* is due to the premature fusion of the metopic suture, often evident because of a longitudinal bone crest in the median frontal region, giving the skull a triangular appearance with hypotelorism and flattening of the lateral frontal regions. *Cloverleaf skull* is due to the premature fusion of the coronal, sagittal and lambdoideal sutures with excessive skull growth in height and to both sides, giving a trilobar appearance. Intracranial hypertension is consistently severe and gives rise to CNS complications.

6.9.1.2 Syndromal Craniosynostoses

Apert syndrome is a rare and serious phenotype described in newborns with acrocephaly and syndactyly of the hands and feet (Fig. 6.9). The premature fusion of both coronal sutures is responsible for acrocephaly, frontal bossing, flat occipital bone, facial dismorphic features (downslanting palpebral fissures, exophthalmus, hypertelorism, small upturned nose, maxillary hypoplasia, low-set ears). Hands and feet present a complete syndactyly (spoon shaped hand) with bone and nail fusion. Synostosis may also found in the carpal and tarsal bones and cervical vertebrae. Mental retardation may be present, as well as intracranial hypertension, particularly in absence of early neurosurgical correction. A complex surgical program must be planned for the correction of multiple

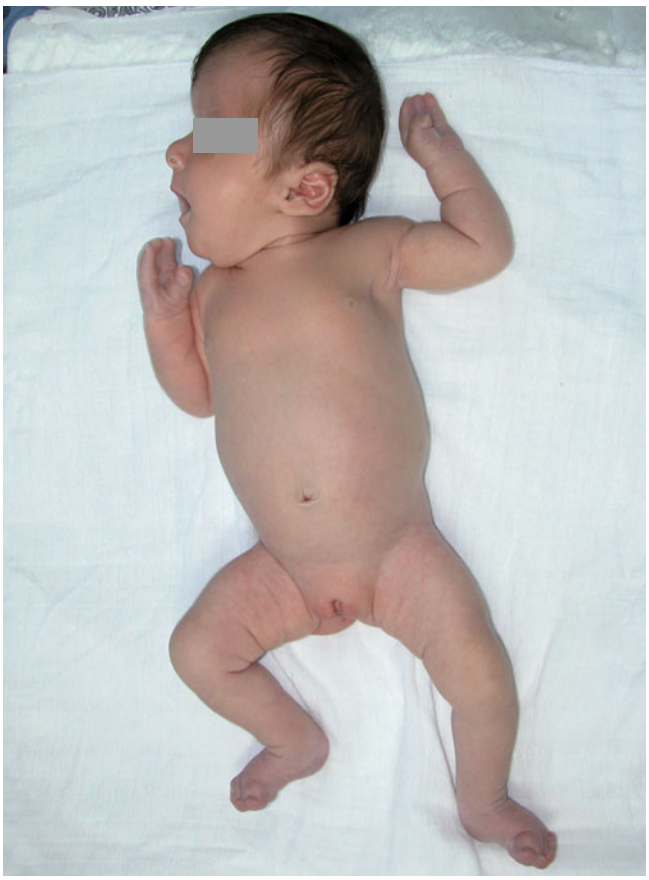

Fig. 6.9 Baby with Apert syndrome showing acrocephaly, frontal bossing, exophthalmus, small upturned nose, maxillary hypoplasia, spoon shaped hands

synostosis (skull remodelling, hand surgery). Apert syndrome is due to mutations in the exon 7 of the *FGFR2* gene (different mutations have been identified), with autosomal dominant inheritance and a high rate of de novo mutations correlated with advanced paternal age [15].

Crouzon syndrome is the most frequently reported syndromal craniosynostosis, characterized by acrocephaly with no hand and foot involvement. Premature fusion of the coronal sutures causes the acrocephaly, frontal bossing and flat occipital bone. Looking at the face, there is hypoplasia of the midline structures, reduced orbital volume and ocular proptosis, strabismus, a small upturned nose, and maxillary hypoplasia. Fusions of cervical vertebrae and mild mental retardation may occur. Crouzon syndrome may be due to different mutations of the *FGFR2* gene with a wide range of phenotypical expression. It may be sporadic, due to a de novo mutation often related to advanced paternal age, or familiar with autosomal dominant inheritance.

Muenke syndrome is a relatively frequent unilateral coronal craniosynostosis with brachydactyly. It is caused by a mutation (Pro250Arg) of the *FGFR3* gene, with autosomal dominant inheritance and variable clinical expression [16]. Most cases are familial, but unless there is an index case in the family, the diagnosis may be missed in newborns with a mild phenotype.

Pfeiffer syndrome is a rare acrocephalosyndactyly caused by several mutations in *FGFR1* and *FGFR2* genes, with autosomal dominant inheritance and variable clinical expressivity. Premature fusion of the coronal sutures causes acrocephaly, frontal bossing and flat occipital bone. Concomitant synostosis of sagittal and lambdoideal sutures may cause a cloverleaf appearance of the skull. The face is characterized by downslanting palpebral fissures, ocular proptosis, strabismus, hypertelorism, maxillary hypoplasia, and low-set ears. Hands and feet usually show hypoplasia of the first ray (large first finger and toe, trapezoidal toe) and various degree of postaxial syndactyly. There may be associated elbow ankylosis or synostosis and vertebral fusions. Most severely affected patients require early surgical correction to prevent CNS complications. The prognosis is related to the degree of phenotypical expression.

6.9.1.3 Treacher-Collins Syndrome (Franceschetti Syndrome)

This syndrome is characterized by a developmental defect of jaw and facial bones. There is wide variability. It is determined by several different mutations in the gene encoding the treacle protein, which has a key role in early craniofacial development [17].

Neonatal features are downslanting palpebral fissures, palpebral coloboma, malar hypoplasia, macrostomia, micrognathia, external ear hypoplasia with atresia of the middle ear and hearing loss. Cleft palate and choanal atresia may also be present. In the most severely affected patients, there may be impaired nutrition and respiratory function. Growth and psychomotor development are usually normal, but affected children need long-term multidisciplinary follow-up. Hearing loss requires early treatment to preserve speech development. Surgical treatment may correct bony defects and achieve useful functional and aesthetic advantages.

6.9.1.4 Nager Syndrome (Acrofacial Dysostosis Nager Type)

A sporadic condition with genetic heterogeneity (both autosomal dominant and autosomal recessive inheritance have been described). Patients have a mandibulofacial dysostosis with associated preaxial limb abnormalities (Fig. 6.10). The mandibulofacial dysostosis is mainly characterized by microcephaly, severe micrognathia and malar hypoplasia, low-set posteriorly rotated ears and external auditory canal atresia. The limb deformities consist of radial aplasia or hypoplasia, radioulnar synostosis with limitation of elbow extension and hypoplasia or absence of the thumbs.

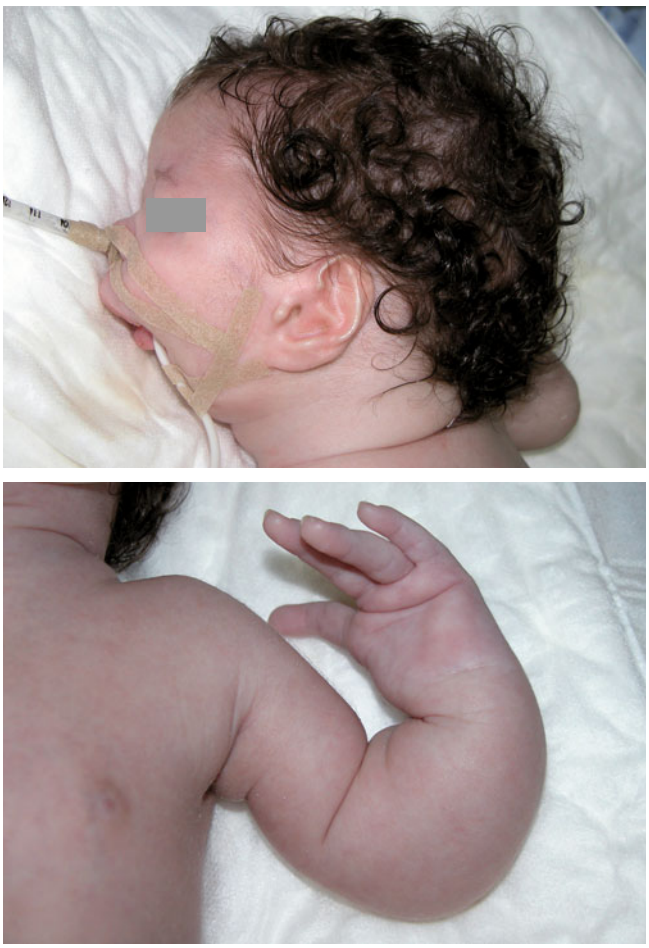

Fig. 6.10 Baby with Nager syndrome showing microcephaly, micrognathia, malar hypoplasia and preaxial limb reduction defects

6.9.2 Thoraco-vertebral Dysostoses

Klippel-Feil anomaly is a developmental defect of the spine, with possible changes at cervical, thoracic and/or lumbar level (short neck, pterygium, kyphosis, scoliosis). X-ray investigation of the spine is required to show vertebral changes (fusions, hemivertebrae, hemispondyls) and complete the diagnostic work-up – there are three clinical variants. It is more frequent in females. Its etiology is heterogeneous, with genetic and environmental (vascular disruptions) causes. It may also be part of a more complex phenotype (cervico-oculo-acoustic syndrome, MURCS association).

6.9.3 Limb Dysostoses

Digital defects are caused by a differentiation defect of one or more contiguous bones. Digital development is determined by a complex genetic system, which is phylogenetically common to most vertebrates. Digital anomalies may be sporadic or familial with Mendelian inheritance, and may be associated with other digital defects (polysyndactyly) and other syndromic defects.

Polydactyly may be defined as the presence of one or more supernumerary fingers or toes. It is termed as complete if it involves all phalanges, partial if it involves only distal phalanges (duplication). In the preaxial forms, the extra digit is related to the thumb; in the postaxial forms it is related to the little finger.

Syndactyly is the fusion of two or more digits: it may involve only skin and muscle or include the bones. In the most severe cases, it may affect all the fingers of the limb causing a "spoon" appearance. *Symphalangism* is the fusion of one or more phalanges in the same digit, with severe ankylosis of the interphalangeal joint.

Brachydactyly is the shortening of a digit due to developmental defect of one or more phalanges. It is often associated with metacarpal or metatarsal hypoplasia.

Ectrodactyly is a severe developmental anomaly of the median axis of the hand or foot causing a "lobster claw" appearance. It is usually caused by a genetic etiology and may be associated with other malformations (e.g., ectodermal defects and cleft palate in the ectrodactyly-ectodermal dysplasia-cleft syndrome [EEC syndrome]).

Oligodactyly is the absence or severe hypoplasia of one or more digital axes. It may be preaxial or postaxial and is frequently associated with other defects.

6.10 Osteochondrodysplasias

A wide and heterogeneous group of genetically determined conditions, involving the development and growth of bony and cartilaginous tissues. The bone involvement is often prenatally diagnosed, although some cases become evident after birth. The overall prevalence at birth is about 1/5000. There has been a significant reduction in recent years because of ultrasonographic prenatal diagnosis of the most severe conditions. The classification of osteochondrodysplasias, based on phenotype, has recently been modified by advances in molecular genetics applied to genes involved the the synthesis of collagen and elastin, fibroblast growth factor receptors, cartilaginous proteins, vitamin D – receptor complex, lysosomal and peroxisomal enzymes.

Lethal osteochondrodysplasias are characterized by death during the perinatal period because of generalized involvement including long bones, spine and cranial bones. Mortality is mainly related to respiratory failure (due to skeletal abnormalities and lung hypoplasia) and associated visceral and CNS malformations. Milder osteochondrodysplasias (with normal life expectancy, short stature and abnormal bone development) may benefit from surgical bone elongation and other corrective surgery and rehabilitation programs.

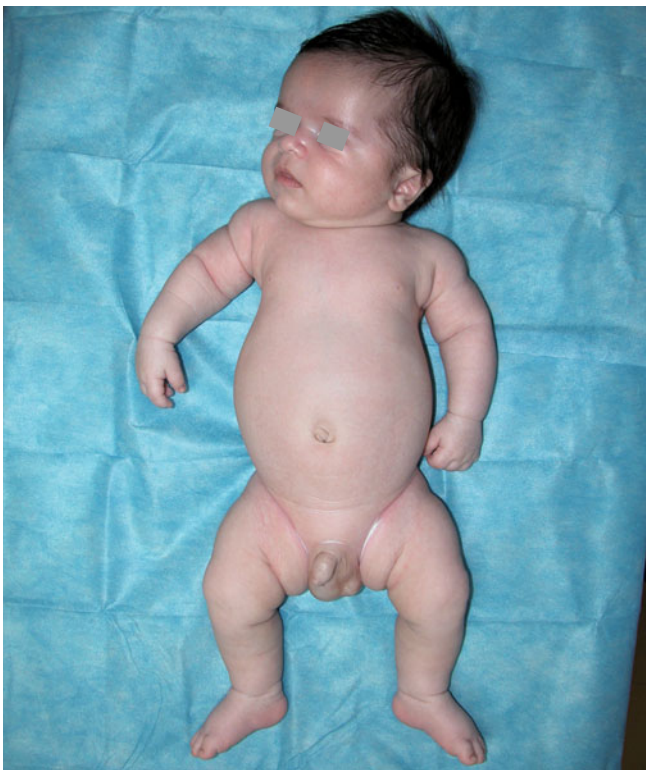

Fig. 6.11 Neonatal achondroplasia with rhizomelic short limb dwarfism, megalencephaly, frontal bossing, facial bone hypoplasia, narrow thorax

6.10.1 Achondroplasia

This is the most common cause of disproportionate short stature with short limbs, determined by a heterozygous mutation (Gly380Arg) of the *FGFR3* gene at 4p16.3. It is inherited as an autosomal dominant trait with a high rate of de novo mutations (80–90% of patients), and is related to advanced paternal age. The recurrence risk is low if parents are not affected. The phenotype at birth (Fig. 6.11) is characteristic: megalencephaly, frontal bossing, depressed nasal bridge, facial bone hypoplasia, prognatism, narrow thorax, rhizomelic short limb dwarfism, brachydactyly, trident hands, hypotonia. Psychomotor development is normal. Patients frequently develop severe orthopaedic complications (lumbar hyperlordosis). In some cases CNS complications, such as the cord compressions and hydrocephalus, may occur during childhood.

6.10.2 Thanatophoric Dwarfism

A severe bone dysplasia with high perinatal lethality, due to a heterozygous mutation of the *FGFR3* gene at 4p16.3. All patients are sporadic and due to de novo autosomal dominant mutations. The phenotype is characterized by severe micromelic dwarfism with bowing and deformations of long bones, "telephone receiver" femurs, narrow thorax, severe platyspondyly (flattening of the vertebral bodies), facial bone hypoplasia, craniosynostosis.

6.10.3 Camptomelic Dwarfism

A severe autosomal recessive bone dysplasia with high perinatal lethality and female prevalence. Due to mutations in the *SOX9* gene at 17q24-25. The phenotype includes sex reversal (female external genitalia with male chromosomes), macrocephaly, large fontanelles, broad depressed nasal root, micrognathia, short neck, pectus carinatum, short limb dwarfism, talipes, anterior bowing of tibiae, poor ossification signs [18].

6.10.4 Diastrophic Dysplasia

A rare autosomal recessive condition due to mutations of the *SLC26A2* gene at 5q32-q33.1. The distinct morphologic abnormality of the growth plate consists of an irregular distribution of degenerating chondrocytes in the resting cartilage with areas of intracartilagenous ossification. Patients have rhizomelic short limb dwarfism, bilateral clubbed foot, cystic lesions of the pinnae with calcification of the cartilage, premature calcification of the costal cartilages, kyphoscoliosis, hip contractures, cleft palate. The "hitchhiker" thumb is particularly characteristic and is due to a deformity of the first metacarpal. Intelligence is normal. Short stature with skeletal abnormalities becomes more marked with advancing age.

6.10.5 Pseudodiastrophic Dysplasia

An autosomal recessive condition described by Burgio in patients with a phenotype similar to diastrophic dysplasia but with proximal phalangeal joint dislocation, normal first metacarpal, platyspondyly, tongue-like lumbar vertebral deformities, marked lumbar lordosis without cystic deformity of the helix. The histologic appearance is different from diastrophic dysplasia and no *SLC26A2* mutations have been demonstrated. Most patients die during the first months of life.

6.10.6 Osteogenesis Imperfecta

A genetically and phenotypically heterogeneous group of conditions characterized by frequent bone fractures [19]. Most frequently affected genes are those encoding for type 1 collagen (*COL1A1* on 17q21-q22 and *COL1A2* on 7q22) with autosomal dominant (AD) and autosomal recessive

(AR) inheritance. Four different clinical variants have been differentiated, but only type II and type III present at birth with a severe phenotype and high perinatal lethality:

- type I (AD, triangular face, blue sclerae, otosclerosis with secondary hearing loss, mitral valve prolapse, macrocephaly, joint hyperlaxity, possible dental abnormalities);
- type II (AD/AR, ossification deficiency, craniotabes, pseudohydrocephalic skull, micrognathia, small nose, blue sclerae, cardiac valve degeneration, endocardic and aortic microcalcifications, multiple dental abnormalities);
- type III (AD/AR, blue sclerae, hydrocephalus, cortical atrophy, joint hyperlaxity, shortness and bowing of limbs, possible dental abnormalities);
- type IV (AD, macrocephaly, frontal bossing, hearing loss, joint hyperlaxity, osteoporosis, mild long bone deformations, possible dental abnormalities).

6.10.7 Osteodysplastic Primordial Dwarfism

A group of brachymelic microcephalic dwarfisms with likely genetic heterogeneity and autosomal recessive inheritance. Different clinical variants have been described in patients with a prenatal onset of severe dwarfism (Fig. 6.12), microcephaly, delayed closure of fontanelles, prominent eyes and strabismus, microretrognathia, pointed nose, high arched palate, oligodontia, small low-set dysplastic ears, sparse scalp hair, pectus carinatum, delayed bone age, severe osteoporosis, multiple skeletal abnormalities (hip dislocation, coxa vara, joint contractures, short bowed long bones, small proximal femoral epiphyses, thin diaphyses of long bones, brachydactyly, talipes). Postnatal failure to thrive, mental retardation, sensory defects are consistently present during growth. Life expectancy is reduced.

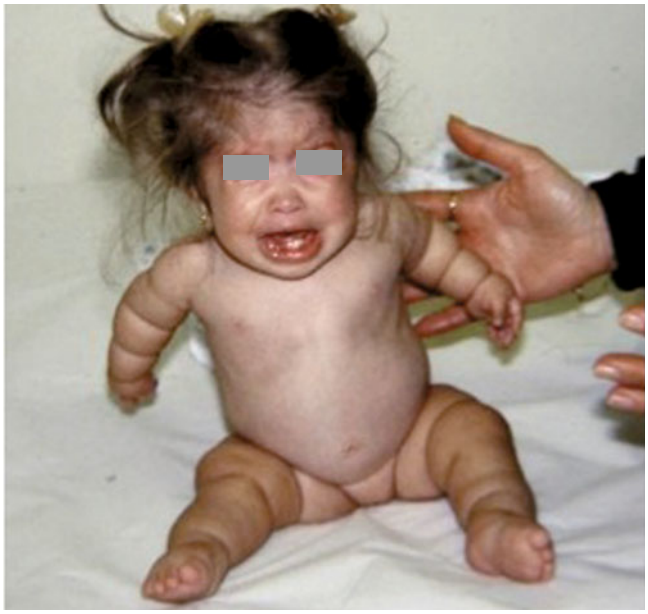

Fig. 6.12 Patient with osteodysplastic primordial dwarfism with microcephaly, severe shortness and deformations of long bones, redundant skin folds at limbs

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7

Prenatal and Postnatal Inflammatory Mechanisms

Christian P. Speer

7.1 Introduction

During the past 15 years, intrauterine inflammation has been identified as an etiologic key factor of fetal and neonatal morbidity and mortality, as well as an adverse long-term outcome of very immature preterm infants. Various microorganisms residing in the maternal recto-vaginal tract may invade the chorioamniotic membranes and eventually induce an inflammatory response in the amniotic cavity, the fetus and the mother [1].

As a consequence of these ascending infections, preterm premature rupture of membranes, maternal amnionitis and preterm birth may ensue. Recent epidemiological data indicate that histologic chorioamnionitis might be much more prevalent in early gestational ages: in approximately 60% of very immature preterm infants of 25–26 weeks' of gestational age histologic chorioamnionitis was present [2], and in nearly half of second-trimester placentas microorganisms were detected within the chorionic plate [3]. In addition, high concentrations of various pro-inflammatory mediators have been identified in amniotic fluid of high risk pregnancies. Besides ascending microbial infections, maternal bacteremia has been shown to initiate a similar sequence of inflammation in the placenta and the amniotic cavity. This intrauterine inflammation may eventually induce a fetal inflammatory response with devastating consequences for the affected preterm infant (Fig. 7.1).

In a number of studies, a strong association between a fetal inflammatory response syndrome and adverse neonatal outcome has been reported. Besides an increased incidence of neonatal early-onset sepsis, more preterm infants developed bronchopulmonary dysplasia (BPD) and brain injury following intrauterine exposure to infection and inflammation. Since the pathogenesis of this intrauterine maternal and fetal disease is only poorly understood, we are currently unable to identify a fetus at risk who might subsequently develop serious sequelae following exposure to prenatal inflammation. Clinical observations indicate that fetal exposure to severe maternal

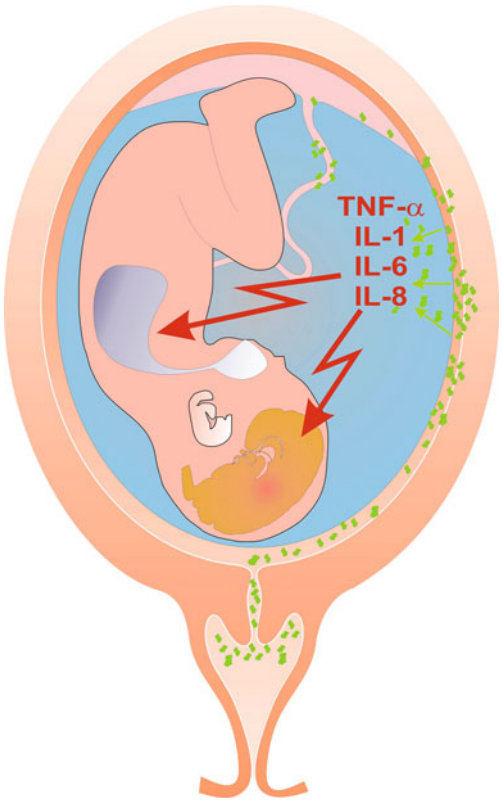

Fig. 7.1 Ascending infection, preterm premature rupture of membranes and maternal amnionitis may result in an intrauterine inflammation reflected by increased intramniotic concentrations of proinflammatory cytokines (TNF-α, IL-1, IL-6, IL-8), which can induce a systemic fetal inflammatory response. There is a clear association between fetal systemic inflammation and adverse neonatal outcome, especially an increased risk of BPD and cerebral injury

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

chorioamnionitis might not necessarily result in an inflammatory response of an individual fetus. In contrast, a rather smouldering or almost unapparent amniotic infection/inflammation might induce a severe fetal reaction.

This article will summarize our current knowledge of pre- and postnatal inflammatory mechanisms in the human fetus and preterm infants in a condensed form, with the main emphasis on acute and chronic inflammatory events in the preterm infant's lung. This aspect has been reasonably well studied, and has been continuously discussed and updated [4–7]. BPD is an evolving process of lung injury, which may affect normal alveolarization and pulmonary vascular development in preterm infants with a possible lifelong impairment of lung function and other developmental consequences.

7.2 Intrauterine Infections/Inflammation and Fetal Involvement

Animal experiments indicate that intraamniotic endotoxin (lipopolysaccharide [LPS]) initiates inflammation in various placental cell types by signaling via Toll-like receptor 4 (TLR4), which subsequently activates nuclear factor κB and a cascade of inflammatory mediators [8, 9]. In fact, various pro-inflammatory cytokines, inflammatory cells and other inflammatory factors have been detected in amniotic fluid and placental tissue of pregnant women at risk. In addition, the presence of proteomic biomarkers characteristic of inflammation in the amniotic fluid was associated with an increased fetal inflammatory response at birth [10]. Moreover, endothelial cell activation with up-regulation and shedding of cell adhesion molecules was present in histologic chorioamnionitis and fetal funisitis [7].

Increased concentrations of pro-inflammatory cytokines in fetal cord blood indicating a systemic inflammatory response during chorioamnionitis were identified as independent risk factors for BPD and brain injury [4–7, 11, 12]. A pronounced infiltration of inflammatory cells, an increased expression of cytokines and large number of apoptotic airway cells have been observed in lung tissues of stillborn human fetuses with funisitis that have been exposed to chorioamnionitis [4].

The potential role of *Ureaplasma urealyticum* (Uu) in fetal disease is currently unclear. Uu is the microorganism most frequently isolated from the amniotic fluid in preterm births and a predominant pathogen detected in the airway secretions immediately after birth [13]. In the Alabama preterm birth study, Uu and *Mycoplasma hominis* were detected in the cord blood of 23% of very immature preterm infants which were more likely to have a fetal systemic inflammatory response syndrome and probably BPD [13].

In animal experiments, antenatal Uu infection contributed to a prolonged pro-inflammatory response, early fibrosis, and changes in morphology and lung function in the immature lung [14].

The fetal inflammatory response to intrauterine infection may also play a crucial role in the pathogenesis of preterm infants' brain injury. The inflammation signal is likely transmitted across the blood-brain barrier and initiates a neuroinflammatory response which can persist over a period of time and sensitize the brain to subinjurious insults in the postnatal period [11, 12].

7.3 Postnatal Factors Inducing Pulmonary Inflammation

7.3.1 Infection

Early-onset sepsis and systemic nosocomial infections have clearly been identified as individual risk factors for BPD. Besides direct adverse effects of microbial pathogens on pulmonary tissues, activated inflammatory cells and mediators may affect the integrity of endothelial and bronchoalveolar cells and may induce an inflammatory sequence which will be described in more detail below. In addition, vasoactive prostaglandin mediators released during septicemia have been shown to prevent ductal closure or to induce reopening. Interestingly, the presence of Uu in the respiratory tract of preterm infants, even without clinical or laboratory signs of infection, was correlated with elevated cellular and molecular markers of inflammation and has been associated with an increased risk of BPD [4].

7.3.2 Mechanical Ventilation

Many *in vitro* studies and animal experiments clearly confirm that any ventilatory trauma of the immature lung may be injurious to airways and lung tissue.

Over-distension of the lungs or cyclic opening and closing of lung units causes disruption of structural elements and release of pro-inflammatory mediators with a subsequent neutrophil influx [4–7]. However, certain ventilatory strategies may cause more damage than others. The strongest inflammatory reaction was observed in those ventilatory strategies with high peak pressure and no positive end-expiratory pressure. "Priming" of the fetal lung by intrauterine endotoxin or exposure to pro-inflammatory cytokines generated during chorioamnionitis is most likely a considerable pathogenetic factor in the initiation of the pulmonary inflammatory sequence. As a consequence, basically each form of mechanical ventilation may act as a "second strike" and may amplify or aggravate the inflammatory reaction in the immature lung [4–7]. Moreover, it has been convincingly demonstrated that even a relatively "traumatic" bag and mask resuscitation immediately after birth causes disruption of structural elements with release of pro-inflammatory mediators.

7.3.3 Hyperoxia

Very immature preterm infants are particularly susceptible to hyperoxia since the activity of antioxidant system is much lower compared to term newborns. These babies have profound deficiency in antioxidant enzyme activity and a very high risk of suffering from potential detrimental effects of hyperoxia and hyperoxemia during the first weeks of life. In various experimental settings, hyperoxia has been shown to be a strong and independent inducer of various inflammatory cells and mediators. Oxidative stress affected a complex orchestra of genes involved in inflammation or extracellular matrix turnover, and the majority of pro-inflammatory genes were up-regulated [4–7].

7.4 Cellular and Humoral Mediators of Inflammation

7.4.1 Neutrophils and Macrophages

Neutrophils, monocytes and macrophages play a central and crucial role in pre- and postnatal inflammatory events. Immediately after initiation of mechanical ventilation, a neutrophil influx into the airways was observed in animals as well as preterm infants, and this reaction was associated with a decrease in the number of circulating neutrophils. This phenomenon was shown to correlate with the extent of pulmonary edema formation which reflects an injury of the alveolar-capillary unit. Circulating neutrophils and monocytes become rapidly activated within 1–3 hours following the initiation of mechanical ventilation as reflected by CD 11b expression. Most likely, activated neutrophils adhere to the endothelium of the pulmonary vascular system and thus initiate a sequence of pathogenetic events. Activated macrophages secrete numerous cytokines and pro-inflammatory mediators which orchestrate the inflammatory response, particularly neutrophil recruitment. Several investigators have convincingly demonstrated that preterm infants with BPD had much higher and persisting numbers of neutrophils and macrophages in their bronchoalveolar lavage fluid than did infants who recovered from respiratory distress syndrome (RDS) [4–7].

Since apoptosis of inflammatory neutrophils and their timely removal by resident macrophages are critical to the resolution of inflammation, neonatal neutrophils which seem to have a prolonged survival may perpetuate inflammatory processes [4–7].

7.4.2 Pro- and Anti-inflammatory Cytokines

Pro-inflammatory cytokines are the most important mediators in the initiation of pre- and postnatal inflammatory response and in the evolution of inflammation. Tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6 and IL-8 are synthesized by various chorioamniotic and inflammatory cells upon stimulation by microorganisms, LPS, and other bacterial cell wall products already in utero or following postnatal infections or biophysical factors. Pro-inflammatory cytokines do not cross the placenta. Increased protein levels and high mRNA expression of pro-inflammatory cytokines have been demonstrated in airway secretions, bronchoalveolar and pulmonary cells, and in the systemic circulation of infants with evolving BPD. For example IL-1 induces IL-8 expression of epithelial cells via a nuclear transcription factor (NFκB)-dependent pathway. IL-8 is involved in the initiation of cellular-endothelial interactions and is probably the most important chemotactic factor in the lung. Recently, a transient over-expression of IL-1 in rat lung by adenoviral gene transfer was accompanied by a local increase in TNF-α expression and a vigorous inflammatory reaction with profound tissue injury and fibrotic changes. Moreover, perinatal expression of IL-1β in pulmonary epithelial cells of a bi-transgenic mouse model induced a lung disease which was clinically and histologically similar to BPD in very immature preterm infants. The presence of TNF-α positive macrophages in lung tissue of preterm infants who had died of severe RDS was strongly associated with a loss of endothelial basement membrane and a destruction of interstitial tissue [4–7]. Experimental and clinical data convincingly indicate that the profound pro-inflammatory cytokine response present in the airways and pulmonary tissue of preterm infants may reflect an inability to regulate inflammation through an adequate expression of anti-inflammatory cytokines such as IL-10 and others. Especially in preterm infants exposed to chorioamnionitis or affected by fetal inflammatory response syndrome, an insufficient inhibition of a high fetal pro-inflammatory cytokine response shortly after birth may increase the risk of BPD [15].

7.4.3 Oxygen Radicals and Proteolytic Mediators

Exposure of preterm infants to high oxygen concentrations can induce direct oxidative cell damage through increased production of reactive oxygen species [16]. Oxygen radicals are not only released by neutrophils and macrophages at sites of inflammation, but are also generated under hyperoxic conditions by free iron or by the cell-bound xanthine oxidase system. Reactive oxygen species exert direct toxic effects on bronchoalveolar structures by induction of inflammation, lipid peroxidation, oxidative inactivation of protective antiproteases and up-regulation of metalloproteinases [4–7]. Animal experiments indicate that oxidative stress is a very early and potent factor in the initiation of pulmonary inflammation [9]. Most importantly, the activity of antioxidant enzymes in

preterm infants is much too low to sufficiently combat oxidative injury. Preterm infants have a profound deficiency of antioxidant activity prior to delivery, immediately after birth and during the first weeks of life, which is the most vulnerable period for an oxidative injury since many babies at risk are receiving high inspiratory oxygen concentrations and are most likely to be exposed to hyperoxia.

At sites of inflammation neutrophils and macrophages release various potent proteases such as elastase, metalloproteinases and others, which play a predominant role in the destruction of the alveolar-capillary unit and the extracellular matrix as well as in remodelling throughout all stages of lung development. In general, data from *in vitro* studies, animal experiments and clinical observation in preterm infants indicate that an imbalance between proteases and protease inhibitors may essentially contribute to lung injury and the pathogenesis of BPD. For example, an imbalance between elastase, a powerful neutral protease, and α_1-proteinase inhibitor has been demonstrated within the airways of preterm infants. α_1-proteinase inhibitor was shown to be functionally inactivated by reactive oxygen species. As a result of elastolytic damage, increased concentrations of various markers of tissue destruction have been identified in airway secretions and urine of preterm infants. This is of particular concern in light of studies showing a markedly reduced alveolar septation in the lungs of infants with BPD [4–7].

Numerous inflammatory mediators and cells have detrimental effects on microvascular integrity. Increased alveolar-capillary permeability is pathognomonic for the early and later stages of inflammation and is clearly associated with a deterioration of lung function. Protein leakage into the alveoli and airways of preterm infants takes place within one hour after initiation of mechanical ventilation. At a postnatal age of 10–14 days, preterm infants who later developed BPD had high concentrations of albumin and other serum proteins in their airway secretion, a phenomenon that contributes to edema formation, to inactivation of the surfactant system and worsening the lung function. Magnetic resonance imaging in infants with BPD confirmed an increased water content and a gravity-induced collapse of the lung [4–7].

7.5 Conclusions

Intrauterine infection and inflammation characterized by chorioamnionitis and a fetal inflammatory response syndrome play a crucial role in the pathogenesis of fetal organ injury. Fetal exposure to inflammatory cells, cytokines and other pro-inflammatory mediators can initiate and induce a pulmonary inflammatory response which might directly affect the alveolar-capillary unit and tissue integrity. It may also be associated with various postnatal factors such as infection, oxygen toxicity and mechanical ventilation and may contribute – at least in part – as a multiple-hit scenario to the development of BPD. As a devastating consequence of the continuous inflammatory process, normal alveolarization as well as vascular development can be compromised with lifelong consequences for the preterm infant. Similarly, a neuroinflammatory response in the fetal central nervous system secondary to fetal infection and/or systemic inflammation is a likely injurious pathomechanism for the developing brain.

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8

The Fetus at Risk: Chorioamnionitis

Mikko Hallman and Tuula Kaukola

8.1 Introduction

Infection and inflammatory injury are the major threats during pregnancy. The extraembryonic tissues play important roles in protecting the fetus. From 10–12 days post conception (p.c.), yolk sack and allantoic vessels provide nutrition to the embryo. By 20 days conception p.c. fetal vessels are discernible in developing villi. They communicate with the embryo via a connecting stalk that later develops into the umbilical cord. By 40 days p.c. the embryo is completely surrounded by the amniotic cavity and attached via the umbilical cord to the hemo-monochorial placenta [1]. Upon complete adherence of the fetal membranes to the decidua at about 20 weeks of gestation, bacterial inflammation may directly penetrate the chorioamnion from any direction and contaminate the amniotic fluid [2, 3]. In high risk pregnancies the protective fetal chorioamnion and placental villi are challenged in multiple ways. These tissues respond by inflammation, producing mediators that may promote labor or affect the fetus in multiple ways. This chapter focuses only on the consequences of chorioamnionitis in the fetus comparing the affected fetuses to the gestation controls.

8.2 Diagnosis and Incidence of Chorioamnionitis

Histologic chorioamnionitis (HCA) is characterized by mostly polymorphonuclear neutrophil (PMN) infiltration of the placenta, fetal membranes and/or umbilical cord. Pathological exam of the placenta, fetal membranes and umbilical cord defines chorioamnionitis (CA) [1, 4]. In most cases HCA is a silent condition, not presenting any detectable symptoms in the mother. However, it is a sign of activation of the immune defense associating with inflammatory activation of preterm labor. Chorioamnionitis is a well accepted risk factor of spontaneous premature birth.

Analysis of a marker of PMNs in amniotic fluid provides accurate diagnosis of CA [5]. The levels of interleukin-6 (IL-6), IL-8 and tumor necrosis factor-α (TNF-α) have been found to be high in the amniotic fluid [6]. However, amniocentesis is not indicated for diagnosis of CA. After birth, the inspection of the placenta, its vessels and the fetal membranes helps to understand the pregnancy outcome. Placentas with severe chorioamnionitis reveal marginal hemorrhages and the amniotic surfaces are covered with invisible white leukocyte-enriched film. Histological specimens are recovered from three sites: chorionic plate, fetal membranes as a roll from the site of spontaneous membrane rupture and segments of umbilical cord [1, 4]. Histological chorioamnionitis is often classified into three grades: (1) mild (5–10 neutrophils per high power field [HPF]); (2) moderate (11–30 neutrophils/HPF); (3) severe (>30 neutrophils/HPF). Involvement of all compartments is studied separately. Chorioamnionitis is additionally defined on the basis of the distribution of inflammation.

Clinical chorioamnionitis presents as maternal fever, foul-smelling vaginal discharge, uterine tenderness and laboratory findings, e.g., leukocytosis or elevated C-reactive protein. In the era of early treatment of infections, two positive criteria (one clinical, one chemical/ laboratory) establish diagnosis. In severe clinical CA, elective delivery is considered for fetal indication.

Prelabor rupture of fetal membranes (PROM) exposes the intra-amniotic space to ascending infection and is mostly associated with CA. In several reports, the cases of preterm PROM are considered under CA or clinical CA, although the diagnosis of HCA is not confirmed. This complicates the comparison of the outcomes between different studies.

Histologic CA is evident in many preterm births (PTB) after spontaneous onset of labor and only in 2–10% of term births (Table 8.1). Although the association between HCA and

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Table 8.1 Prevalence of pregnancy conditions and incidence of chorioamnionitis (HCA) in pregnancy conditions

Pregnancy condition	Prevalence % of all births	HCA % in given pregnancy condition
Term	85–92	2–10
Post-term	1–5	5–15*
Preterm	5–13	15–25
– Spontaneous onset	3–9	25–35
PROM	1.5–4	50–80
32–36 weeks	2–7	20–30
28–32 weeks	0.6–1.2	30–50
<28 weeks	0.3–0.6	40–70
– No spontaneous onset	2–4	5–15

* Meconium in amniotic fluid (50%) associates with green staining of chorioamnion that contains meconium-laden macrophages; associated chorioamnionitis suggests infection.
The ranges of the incidence figures are based on population studies that, however, may not be representative [25, 38, 42]. The reported incidence figures are likely understated, since more extensive examination of fetal membranes reveals a higher incidence of inflammatory cells [43].
In preterm PROM, labor nearly always starts spontaneously.

spontaneous PTB is strong, neither the specificity nor the sensitivity is very high. In near-term births due to spontaneous onset of labor, HCA is less frequent (10–25%) than in extremely preterm spontaneous births (50–80%). Yet, even in elective term births without labor, bacterial organisms may be present [7].

Fetal inflammatory response syndrome (FIRS) is defined as an increase in IL-6 in cord blood in CA [8]. Funisitis is another diagnostic criterion of FIRS. Although FIRS is associated with an increased risk of early postnatal infection, most of the premature newborn affected by FIRS (> 90%) have negative blood cultures. According to a regional cohort of 163 preterm Finnish infants, born before 32 weeks of pregnancy, 50% of HCA cases had FIRS on the basis of funisitis, elevated cord IL-6, or both.

8.3 Antenatal Findings in Chorioamnionitis

In term pregnancies, maturation and shortening of the cervix and rupture of fetal membranes expose the amniotic space to microbes. In the setting of spontaneous preterm labor, the inflammatory mediators in general play a much more prominent role in the activation of the labor process than in term labor. Histological inflammation may persist for a long period during pregnancy as a condition with apparently little consequence. It has been postulated that after mid-pregnancy when the expanding fetal membranes seal the endometrial cavity, the incidence of ascending inflammation becomes rare [9].

Besides microbes and endotoxins, other triggering agents (host *vs* graft reaction, meconium) may play a role in the etiology. In HCA, the amniotic fluid or fetal membranes reveal microbes that, however, may also be detected in elective births without labor using molecular probes specific for bacteria [6, 7, 9]. The most common microbe is *Ureaplasma urealyticum* (20–40%) (Table 8.2). The traditional pathogens (Group B Streptococci, *E. coli*, Enterococci, *Listeria monocytogenes*) are not very common (2–10%).

The ascending route is the most common in HCA and bacterial vaginosis (BV) is a risk factor. In BV, the incidence of lactobacilli as a normal vaginal inhabitant has decreased at the expense of other microbes, including *Gardnerella vaginalis*, *Mobiluncus* spp., anaerobic gram-negative rods, *M. hominis* and *Ureaplasma urealyticum*. Most of microbes in BV are frequently detected in HCA. However, BV predicts the risk of preterm birth with a low specificity [10]. Periodontitis is another proposed risk factor of spontaneous PTB [11]. Antibiotics

Table 8.2 Fetal consequences of chorioamnionitis or chorioamnionitis with complicating event. Comparison to infants born preterm with similar gestation and without evidence of chorioamnionitis

Postnatal morbidity	Histologic chorioamnionitis	+Fetal inflammation	+Clinical chorioamniontis	+PROM
Infection	↑	↑↑	↑↑	↑↑
RDS	↓	~* or ↓	~* or ↓	~* or ↓
BPD	~ *	–	–	~ or ↑*
Severe RDS	~* or ↓	–	–	↑
IVH	~ or ↑*	~ or ↑	↑	↑
CP	~or ↑*	–	↑	↑

* Progress in antenatal and neonatal treatment practices (antenatal glucocorticoid and gentle neonatal cardiopulmonary treatment practices) have decreased the serious morbidity. Explanations: (~) both lower and higher incidences reported; (–) reports nonexistent or scanty.

have not been beneficial in prolonging the overall duration of high-risk pregnancy without PROM according to available meta-analyses. After preterm PROM, antibiotic prophylaxis (macrolide ± additional antibiotics *vs* expectant management) increases the duration between PROM and birth [12].

8.3.1 Specific Infections Associated with Chorioamnionitis

Placental villi are affected in infections causing intrauterine growth restriction [13]. *Treponema pallidum* causes hypercellular villitis with mononuclear infiltration, sometimes necrotizing funicitis or plasma cell rich CA. Intrauterine candidiasis is associated with clusters of characteristic structures, surrounded by inflammatory cells in umbilical cord, placenta and fetal membranes. Typical placental findings of severe *Cytomegalovirus* infection include plasmacytic villitis, thrombosis of villous capillaries, necrosis of villous tissues, and inclusion-bearing cytomegalic cells. *Toxoplasma gondii* is associated with lymphoplasmacytic villitis and sclerosis, along with toxoplasma cysts in amnion/chorion. Enteroviruses, mumps, and rubella cause distinct placental inflammatory lesions. *Listeria* infection causes acute necrotizing villitis and CA. Early-onset Group B Streptococci (GBS) causes CA and amniotic fluid is a growth media of GBS. The high risk mothers are frequently heavily colonized with GBS (vaginal flora, urinary tract, breast milk). In these cases early neonatal GBS is effectively prevented by intrapartum administration of antibiotics. The recurrence rate of the carrier status is high (see Section X: Fetal and Neonatal Infections).

Ureaplasma urealyticum is the most frequently isolated microbe in CA. *Ureaplasma* spp. may cause pneumonia and occasionally bacteremia and meningitis in susceptible newborn infants. According to randomized trials, erythromycin treatment against *Ureaplasma* species prolonged the duration of pregnancies after preterm PROM. Further randomized trials are required to demonstrate that appropriate antimicrobial treatment of the high-risk infants eradicates *Ureaplasma* spp. and decreases the risk of BPD [14].

The causes of increased susceptibility in some pregnancies to CA remain to be studied. Genetic factors may influence the individual susceptibility, as some mutations or alleles cause defects in innate immunity or failures of appropriate antibody formation. Environmental risk factors warrant further investigation and development of vaccines against specific pathogens would offer a tool for prevention.

8.3.2 Inflammation without Microbes

Villitis of unknown etiology (VUE) is found in 5–15% of predominantly term placentas. It is associated with intrauterine growth restriction, fetal death and perinatal asphyxia. In VUE, CD3-positive maternal T lymphocytes gain access to the villous stroma. Fetal antigen-presenting cells (Hofbauer cells) expand and express class II major histocompatibility complex molecules. Maternal monocyte-macrophages in the perivillous space likely amplify the immune response. VUE may represent a state combining maternal allograft rejection and graft *vs* host disease mechanisms [15, 16].

Meconium that is prevalent in post-term (20–70%) and term (5–15%) pregnancies, associates with green staining of chorioamnion containing meconium laden macrophages and occasionally chorioamnionitis.

8.4 Chorioamnionitis as a Risk Factor for Acute and Chronic Diseases

It has been proposed that the ascending inflammation is mainly distributed transcellularly via amniotic fluid. On the other hand, in clinical chorioamnionitis transplacental spreading prevails. According to experimental studies and clinical observations, placental circulation is adversely affected as a result of transplacental inflammatory insult [17], whereas the transcellular spreading of inflammation acutely boosts the host defense [18, 19]. The fetal consequences of CA are additionally dependent on severity and etiology of the inflammation, as well as multiple, poorly understood genetic and environmental risk factors. Amniotic fluid, contaminated with inflammatory cells, cytokines and/or microbes, is swallowed as well as exposed to developing airways during fetal breathing movements. Hematogenous spreading by direct invasion via placental villi or umbilicus to the fetal compartment is another route. CA is not always diagnosed by histology. Defining CA biochemically (inflammatory cytokines), microbiologically or clinically will alter the target population and hence the associated risk [20].

8.4.1 Experimental Data

Introduction of bacterial endotoxin (LPS) or proinflammatory cytokine (IL-1) to amniotic fluid evokes an acute maturation of the surfactant system [18, 19]. This cytokine-induced pulmonary epithelial differentiation enhances the capacity of premature neonatal respiratory adaptation. Its effect is additive with that of glucocorticoid [19]. LPS or cytokine (e.g., IL-8) induces acute airway inflammation that may spread systemically [21]. Antenatal glucocorticoid protects the preterm fetus affected by FIRS against vascular insults, stabilizing the blood pressure and strengthening endothelial and epithelial barriers in blood vessels after premature birth. In pregnant rodents, a high dose of LPS to the maternal compartment induces an acute fetal perfusion defect, illustrated by increased

resistance in placental villous perfusion and resulting in low-output fetal cardiac failure. According to animal model data, acute transplacental inflammatory insult may result in brain injury [22]. Inflammation may gain access into the immature brain as specific chemokines serving as ligands for their receptors in the target tissue or promoting neutrophil-mediated breakdown of blood-brain-brain barrier [23]. Intracerebral cytokine storm promotes free radical attack, necrosis, apoptosis and brain injury. Immature oligodendrocytes are especially vulnerable (see Chapter 128).

8.4.2 Pulmonary Consequences of CA

The reports of premature patients from the early 90s associate CA mostly with adverse outcomes. In these studies, clinical CA was overrepresented, antenatal glucocorticoid was rarely given, and advanced neonatal respiratory care were not available. The early study of Watterberg revealed an association between CA and a low risk of RDS and a high risk of BPD as studied in 53 preterm infants [24]. The risk of BPD associated with increased tracheal IL-1β levels [24]. Later reports representing the era of antenatal glucocorticoid and surfactant therapy confirm a HCA-associated substantial decrease in the risk of RDS, and no increase in the risk of BPD [25–27].

Recent cohort studies with larger populations have additionally found that in very preterm infants the high proinflammatory cytokines detected in cord blood in HCA often *decrease* shortly after birth and do not associate with adverse pulmonary consequences [26, 27]. However, within 24 hours after a very preterm birth, very high levels of specific cytokines, many from non-CA pregnancies, associate with both RDS and BPD [26]. Modest inflammatory response in CA may additionally be due to immune paralysis that associates with antenatal glucocorticoid treatment [28] or complicates prolonged PROM [29]. Structural immaturity of the lung after extremely preterm birth predisposes to "new" BPD, suggesting that inhibition of angiogenesis and of formation of true alveoli play important roles in the pathogenesis BPD [30]. Fetal inflammation exposes the lungs to a lower risk of growth failure and lack of alveolarization than inflammation or recurrent infections after extremely preterm birth. It is possible that environmental factors, such as high oxygen tension after birth, plays an important interactive roles in pathogenesis. In infants born after 32 weeks of pregnancy, the development of lung structure is more advanced and the risk of BPD is very low, despite serious acute neonatal lung disease.

8.4.3 Neurological Consequences

Half of all children with CP are born at term (CP prevalence 1–1.5 per 1000 term born) including mostly cases of hemiplegia and quadriplegia, whereas in very preterm infants diplegia predominates and CP cases are fairly common in this population (4–8 per 100 preterm) [31]. The pattern of cord blood immunoproteins predicting CP is different in very preterm and term infants [32].

According to a large population based cohort, the incidence of CP among term and preterm infants has remained stable, or even declined, since the 70s, suggesting that advances in treatment practices influence the long-term outcomes [31].

8.4.4 Infants Born Very Preterm

In many but not all studies, CA is a risk factor of IVH among infants born very preterm [33–35]. Despite common use of antenatal glucocorticoid and more gentle neonatal treatment practices, decreasing the risk of IVH, CA has been linked to IVH, emphasizing the role of immunological activation in fetus as a pathway to cerebral injury before birth. In populations mostly representing *clinical* CA, the risk of revere IVH is increased in preterm infants [36]. There is also evidence of increased risk of CP and cognitive problems in preterm infants born in clinical CA [37]. Besides clinical CA, cases of "silent" CA with an additional defect in placental perfusion are associated with abnormal neurological outcome [33]. However, infants born with HCA as a whole do not have a higher frequency of neurodevelopmental problems than the gestation controls [38]. More prospective cohort studies are underway.

8.4.5 Infants Born Near-Term to Term

A case-control study of a cohort of ~200,000 infants born at 36 weeks or later in gestation during the years 1991 and 1998 revealed CA or endometritis in 14% of CA cases and 4% of random controls, translating CA as an independent risk factor (OR 3.8, CI 1.4–9.3) of CP [39], consistent with other studies. Clinical CA and maternal fewer were associated with increased risk.

The diagnosis of silent HCA covered only less than 25% of the CA population and there was no detectable association between silent HCA and CP. In a meta-analysis a significant association was found both between clinical CA and CP (RR 1.9, 95% CI 1.5–2.5) and between clinical CA and cystic periventricular leukomalacia (RR 2.6, 95% CI 1.7–3.9) [40].

Quadriplegia as a separate trait in term and near-term neonates was associated with severe hypoxic-ischemic insult. In contrast, hemiplegia is associated with brain infarction, and one risk factor of brain infarction was preterm PROM [41].

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9

Diagnosis of Fetal Distress

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9.1 Fetal Distress

Fetal distress is a very broad term, which can be used in many clinical situations. Although it is difficult to give a precise clinical definition, obstetricians usually use this term to indicate that the fetus is becoming hypoxic. Immediate delivery has to be considered, because neurological damage may occur when the fetal brain is deprived of oxygen.

The diagnosis of fetal distress based upon heart rate is imprecise because heart rate patterns are only a reflection of the efficiency of physiological mechanisms which depend on blood flow and oxygenation. Furthermore, activity of this control mechanism is influenced by the pre-existing state of fetal oxygenation as with chronic placental insufficiency. Therefore, heart rate patterns are now described as "reassuring" or "non reassuring": in the case of a "non reassuring" heart rate pattern, there is a risk of fetal distress.

The recognition of risk and the knowledge of appropriate measures to treat fetal distress are of the utmost importance. Antepartum fetal testing is used to assess hypoxia in high-risk pregnancies, and monitoring during labor supplies information on the status of the fetus prior to birth.

9.1.1 Fetal Oxygenation

9.1.1.1 Physiology

Oxygenation may be defined as the process of transporting molecular oxygen from air to the tissues of the body. In the fetus this first involves oxygen transfer across the placenta; then there is reversible binding of oxygen to fetal hemoglobin and transport in the fetal blood stream; and, finally, oxygen consumption for growth and metabolism. Energy is derived from the combination of oxygen and glucose to form carbon dioxide and water [1]. Removal of carbon dioxide and protection against acidosis is the reverse mechanism of oxygen delivery and is helped by the rapid diffusion, high solubility and volatility of carbon dioxide. In the adult, carbon dioxide is excreted in the lungs while bicarbonate and hydrogen ions are removed by the kidney. In the fetus, both these functions are carried out by the placenta. When there is inadequate oxygen supply, the Krebs cycle cannot operate and pyruvate is converted to lactic acid. This enters the blood and there is a systemic acidosis, unless it is either metabolized or excreted. The amount of oxygen bound to hemoglobin is not linearly related to oxygen tension (pO_2). Each type of hemoglobin has a characteristic oxygen dissociation curve which can be modified by various factors, such as pH and the concentration of 2,3-diphosphoglycerate (2,3-DPG). For example, when 2,3-DPG rises in response to anemia or hypoxia, it binds to and stabilizes the deoxygenated form of hemoglobin, resulting in a shift of the oxygen dissociation curve to the right and therefore release of oxygen to the tissues. Although *in vitro*, both adult (HbA) and fetal (HbF) hemoglobins have the same oxygen dissociation curves, adult human blood has a lower affinity for oxygen than fetal blood because of its greater binding to 2,3-DPG. The higher affinity of fetal blood for oxygen helps the transplacental transfer of oxygen. Furthermore, since the P50 of fetal blood is similar to the umbilical arterial pO_2, the fetus operates over the steepest part of the hemoglobin oxygen dissociation curve and, therefore, a relatively large amount of oxygen is released from the hemoglobin for any given drop in pO_2 [1].

9.1.1.2 Hypoxia

Fetal hypoxia is a condition characterized by oxygen deficiency in the tissues: any cause that leads to a conversion from an aerobic to an anaerobic metabolism produces less energy and more acid. If the oxygen supply is not restored, the fetus dies.

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Table 9.1 Causes of reduced fetal oxygenation

Maternal	– Respiratory insufficiency – Hypotension – Hypertension – Collagen/vascular disease – Diabetes – Shock – Hemolytic crisis – Asthma/bronchospasm
Placental	– Abruptio placentae – Infarction – Confined mosaicism – Placenta previa
Uterine	– Rupture – Hyperstimulation – Prolonged labor
Fetal	– Arrhythmia – Hydrops – Myocarditis – Congenital abnormality
Umbilical cord	– Funicular loop – Compression – Prolapse – Vasa previa bleeding

Hypoxia may result from:

1. reduced placental perfusion with maternal blood and a consequent decrease in fetal arterial blood oxygen content due to low pO_2 (hypoxemic hypoxia);
2. reduced arterial blood oxygen content due to low fetal hemoglobin concentration (anemic hypoxia);
3. reduced blood flow to the fetal tissues (ischemic hypoxia) [1].

Reduced fetal oxygenation may result from a variety of sources (Table 9.1) [2].

9.2 Clinical Diagnosis (of Fetal Distress)

9.2.1 Antenatal Surveillance

9.2.1.1 Identification of a Fetus at High Risk of Distress

The identification of at-risk pregnancies and their surveillance are currently of high priority in obstetric practice. Fetal distress and antepartum death can be substantially reduced if fetal surveillance is aimed at the early detection of signs of fetal oxygen deprivation. Ultrasound imaging has helped with detection of the vulnerable fetus.

The purposes of ultrasonography studies are: (i) to have accurate pregnancy dating, to diagnose multiple pregnancies and to detect fetal anomalies; and (ii) to monitor fetal growth and to recognize small for gestational age (SGA) and growth restricted (FGR) fetuses who may be more susceptible to the damaging effects of fetal hypoxia.

It is not always easy to decide whether a fetus is SGA as a result of genetic causes or if it suffers from placental insufficiency and malnutrition (intrauterine growth restriction [IUGR]) (Fig. 9.1), but it is important to detect fetuses with an asymmetric profile of growth because these fetuses are at significant risk of hypoxia, acidosis and death. This condition is also a common cause of premature delivery, is associated with increased perinatal mortality and morbidity and is considered synonymous of "chronic fetal distress". The identification of SGA pregnancies allows for monitoring and optimizing the mode and time of delivery.

If IUGR is suspected, it is important to perform an ultrasound biometric evaluation of the fetus as early as possible and then to follow fetal growth with serial and repeated (every 2 weeks) ultrasound investigations.

Before assessing fetal size, it is necessary to make an accurate assessment of gestational age.

Determination of Gestational Age and Assessment of Fetal Size

The accurate assessment of gestational age is very important in everyday clinical practice.

Fetal body measurements reflect the gestational age of the fetus. This is particularly true in early gestation (first and early second trimester). In patients with an uncertain last menstrual period, biometric measurements must be made as early as possible to date the pregnancy accurately. The embryo crown-rump length (CRL) measured obtained by ultrasound, has been shown as the best parameter during the first trimester (accuracy: ± 5–7 days). This measurement can be made between 6 and 13 weeks and gives a very accurate estimation of the gestational age.

The use of multiple parameters (biparietal diameter [BPD]; head circumference [HC]; transverse cerebellum

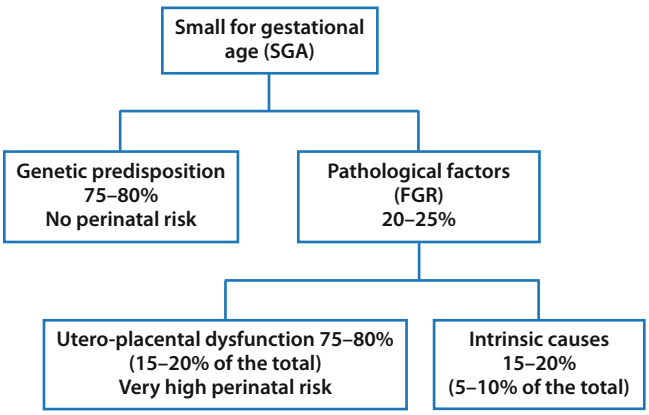

Fig. 9.1 Reduced fetal growth: etiopathogenesis

Table 9.2 Second trimester: accuracy of multiple parameters in the determination of gestational age

Weeks	BPD	HC	FL
12-18	± 1.19	± 1.19	± 1.38
19-24	± 1.73	± 1.48	± 1.80
25-30	± 2.18	± 2.06	± 2.08

Table 9.3 SGA: US diagnostic accuracy

Ultrasound parameters	Sensitivity (range)	Specificity (range)
Abdominal circumference	67–86%	80–92%
Ratio among biometrical parameters	62–82%	80–93%
Fetal weight estimation (FWE)	64–83%	79–96%

diameter [TCD]) is generally recommended for the determination of gestational age in the second trimester (Table 9.2).

To assess if a fetus has normal growth, the following fetal biometry is used:

1. BPD and HC;
2. abdominal circumference (AC), which is the most important measurement in the second half of pregnancy; it reflects fetal size and weight rather than age [3];
3. femur length (FL), which reflects longitudinal growth of the fetus.

Repeated measurement of AC is considered the most sensitive parameter for following fetal growth (Table 9.3). In recent years, three dimensional ultrasound and measurements of fetal subcutaneous tissue have been porposed as methods that may improve the accuracy of fetal size calculations [4, 5].

When the suspicion of IUGR is confirmed, fetal surveillance, placental function and fetal behavior measurements should be initiated and fetal growth should be followed longitudinally with other diagnostic methods (see § 9.3).

9.2.1.2 Identification of Fetal Distress

Fetal distress may be discovered in pregnancy ("antepartum testing") or during labor and delivery ("intrapartum testing") using different approaches.

9.3 Antepartum Testing

There are various tests that allow clinicians to evaluate fetal oxygenation and fetal well-being. These are described below.

9.3.1 Biophysical Profile (BPP)

The integration of aspects of fetal behavior, such as gross body movements, breathing movements and fetal tone, together with cardiotocography (see below) and the amount of amniotic fluid constitute the fetal BPP. Described by Manning, the BPP is based on ultrasound and is widely used for antepartum fetal surveillance [6]. It can be done in the later stages of pregnancy from the beginning of the third trimester. The test is more frequently used in cases where the pregnancy is over 40 weeks to ensure fetal well-being; in some cases it is performed because of the presence of high-risk factors (previous pregnancy loss in the second half of pregnancy, maternal hypertension, diabetes mellitus, IUGR, etc).

BPP has been tested only in high-risk pregnancies, and there is evidence from uncontrolled observational studies that it has a good negative predictive value for fetal death. It is not suitable for fetal surveillance in unselected populations.

BPP is a score based on five parameters: a score of 0 (abnormal) or 2 (normal) is given for each of five categories (Table 9.4).

The second part of the test consists of a CTG evaluation (see § 9.3.2.1).

Table 9.4 Fetal biophysical profile

Biophysical variable	Normal (score = 2)	Abnormal (score = 0)
Fetal tone	1 or more episodes of active extension with return to flexion of fetal limb(s) or trunk (opening and closing of hand considered normal tone)	Slow extension with return to partial flexion, movement of limb in full extension, absent fetal movement, or partially open fetal hand
Gross body movements	2 or more discrete body/limb movements within 30 min (episodes of active continuous movement considered as a single movement)	<2 episodes of body/limb movements within 30 min
Fetal breathing movements	1 or more episodes of ≥20 s within 30 min	Absent or no episode of ≥20 s within 30 min
Qualitative amniotic fluid volume	1 or more pockets of fluid measuring ≥2 cm in vertical axis	Either no pockets or largest pocket <2 cm in vertical axis
Fetal heart rate (FHR)	2 or more episodes of acceleration of ≥15 bpm and of >15 s associated with fetal movement within 20 min	1 or more episodes of acceleration of fetal heart rate or acceleration of <15 bpm within 20 min

Modified from [6].

A score below 6 is not reassuring and is considered borderline. The test may be repeated as often as daily until delivery, although most often it is a one time event, or a weekly event depending on the reason for the biophysical profile.

9.3.1.1 Amniotic Fluid Evaluation

This is an important parameter for the assessment of fetal well-being. Amniotic fluid volume is a reflection of placental perfusion and normal fetal blood flow from the placenta and may be decreased when there are factors causing growth restriction [7].

Although an experienced examiner can assess the adequacy of the amniotic fluid volume, an amniotic fluid index (AFI) has been developed to standardize this assessment. This index is obtained by calculating the sum of deepest pocket of amniotic fluid in each of the four quadrants of the uterus. Another technique is the vertical measurement of the major pocket (Manning technique: cut-off 10 mm).

During the third trimester, normal amniotic fluid correlates with an AFI of 10–20 cm. Borderline values are 5–10 cm for decreased fluid and 20–24 cm for increased fluid depths. Pregnancies with decreased amniotic fluid volume are an indication for additional surveillance and assessment. For some authors, prospective evaluation of amniotic fluid index alone in low-risk pregnancies does not seem to be a good predictor of complications during a pregnancy or the perinatal period [7, 8], but others consider that reduced amniotic fluid volume indicates chronic oxygen deficiency: perinatal mortality in pregnancies with oligohidramnios is 50 times higher than in pregnancies with normal AFI.

9.3.1.2 Amnioscopy

At term, it is possible to visualize the amniotic fluid colour using amnioscopy. If it is clear, the liquor is considered normal; if it is colored, there is a possibility of fetal distress because during fetal hypoxia there is an increase in fetal intestinal peristalsis with the release of meconium from the fetal rectum. This investigation is no longer used as often as it was in the past.

9.3.2 Cardiotocography

Cardiotocography (CTG) is the recording (-graphy) of the fetal heartbeat (cardio-) and the uterine contractions (-toco-). Recordings are done using two separate transducers, one for the measurement of the fetal heart rate and the other for measuring uterine contractions.

The CTG trace shows two lines: the upper shows the fetal heart rate in beats per minute; the lower is a recording of uterine contractions. Interpretation of the results of the monitoring is complex and different patterns can be seen on a CTG.

1. *Baseline rate*. The baseline rate should be between 110 and 150 beats per minute (bpm) and is measured when the fetal heart rate is stable (i.e., without accelerations and decelerations). It should be taken over a period of 5–10 minutes. The rate may change over a period of time but normally remains fairly constant.
2. *Bradycardia*. This is defined as a baseline heart rate of less than 110 bpm. A suspicious bradycardia is defined as being between 110 and 100, whereas a pathological pattern is below 100. A steep sustained decrease in fetal heart rate is indicative of fetal distress.
3. *Tachycardia*. A suspicious tachycardia is defined when the FHR is between 150 and 170 bpm, whereas a pathological pattern is above 170 bpm. Tachycardias can be a response to fever, fetal infection, fetal movements, tocolytic drugs, use of tea or coffee and occasionally a sign of fetal distress (in conjunction with other abnormalities). An epidural may also induce a tachycardia in the fetus.
4. *Baseline variations*. The short-term variations in the baseline should be between 10 and 15 bpm (except during fetal sleep, which should be no longer than 60 minutes). Prolonged reduced variability along with other abnormalities may indicate of fetal distress.
5. *Accelerations*. This is a transient increase in the fetal heart rate of greater than 15 bpm for at least 15 seconds. Two accelerations in 20 minutes are required for the trace to be considered "reactive" i.e., normal. Accelerations are a good sign as they show fetal responsiveness and the integrity of mechanisms controlling the heart rate.
6. *Decelerations*. These may either be normal or pathological. Early decelerations occur at the same time as uterine contractions and are usually due to fetal head compression and therefore occur during the first and second stages of labor with descent of the head. They are normally benign. Late decelerations begin after the start of a contraction and persist after the end of a contraction and suggest fetal distress. Variable decelerations vary in timing and shape with respect to each other and may indicate hypoxia or cord compression.

9.3.2.1 Non Stress Test (NST)

Since its introduction during the 1970s, the non stress test (NST) has remained one of the cornerstones of fetal surveillance. It is based on CTG and it reflects cardio-respiratory regulation of fetal heart: the monitor records fetal heart rate in conjunction with uterine activity.

The purpose of NTS is to try to identify potential fetal compromise as a result of placental insufficiency and hypoxia and to take corrective action [9]. It is used to identify signs of fetal distress in all situations in which pregnancy complications may cause harm to the fetus. It can also be performed as a precaution when there have been problems complicating

a previous pregnancy or in the presence of risk factors (e.g., diabetes, intrauterine growth restriction).

The test is more frequently done between 38 and 42 weeks' gestation; however, it can be used as early as the beginning of the third trimester.

A normal test is defined as a "reactive" pattern that requires a minimum of two accelerations (15 bpm increase from the baseline during 15 s) during a 20 minute test. A reactive trace after 10–20 minutes is generally regarded as sufficient for normality. A "non-reactive" result suggests the possibility of fetal distress, requiring further assessment (biophysical profile, a stress test).

The predictive value of a non-reactive NST is poor when outcome parameters such as fetal death and fetal distress during labor are used [10]. A reactive test can be expected to impose a 10 fold lower risk of intervention for fetal distress during labor than a pregnancy with a non reactive test. However, there are wide observer differences in the qualitative interpretation of the FHR trace and agreement between observers is low, especially for decelerations, which are the most difficult pattern to interpret. For this reason, to eliminate observer variability and to increase the accuracy of CTG, a computerized analysis, was developed by Dawes and coworkers [11, 12]. Nowadays use of the computerized CTG has increased the predictive ability of NST for the diagnosis of fetal hypoxia during the antepartum period compared with the traditional CTG – compared to computer analysis, observers fail to identify 36% of FHR traces with at least one deceleration.

9.3.2.2 Contraction Stress Test (CST)

The contraction stress test may be used after a non reactive NST. This test monitors the response of fetal heart rate to uterine contractions. These contractions may occur on their own or following the administration of oxytocin.

A normal placenta has extra capacity for transporting oxygen, allowing large amounts of oxygen to pass easily from the maternal to the fetal circulation. If the placenta is damaged, less oxygen may cross. When there are contractions, the vessels feeding the placenta are squeezed, limiting the blood flow and oxygen delivery. If the oxygen passage across the placenta drops below a certain point, the fetus responds with a specific type of heart rate deceleration, which occurs after the peak of a contraction and which is known as a "late deceleration" [10].

The contraction stress test is considered "positive" if there are regular late decelerations. When no late decelerations occur, the CST is negative, and this is considered reassuring. A positive CST is a sign that the placenta may not be delivering adequate amounts of oxygen to the fetus. Given a positive CST, a pregnancy at term should be delivered, although this is not necessarily apporpraite for very premature pregnancies.

Because a contraction stress test takes more time to perform than a biophysical profile, it is seldom done these days.

9.3.3 Doppler Examination of Fetal and Placental Circulation

Doppler ultrasound provides a non-invasive method for the study of the fetus's hemodynamic status.

The application of Doppler to obstetrical ultrasound provides new knowledge about fetal circulatory physiology in health as well as in those compromised by chronic hypoxia because of placental insufficiency. This functional assessment of fetal well-being is done by observing fetal movements, breathing, muscle tone and heart rate. Additionally, the volume of amniotic fluid gives an indirect assessment of the pregnancy.

Investigation of the flow patterns of the uterine and umbilical arteries gives information on flow through the uteroplacental and feto-placental circulations respectively. Doppler studies of fetal organs are used to detect the hemodynamic rearrangements that occur in response to fetal hypoxemia, in particular by insonnation of the middle cerebral arteries (MCA) and ductus venosus (D).

The Doppler indices (Table 9.5) indirectly reflect the impedance of the circulation downstream to the point of insonnation, and there is a significant association between abnormal Doppler indices and fetal hypoxia, fetal acidosis, and adverse perinatal outcome.

Doppler ultrasonography is not routinely employed in low-risk pregnancies, but has been extensively used for maternal-fetal evaluation in high-risk pregnancies, contributing to a reduction in perinatal mortality, especially in the management of IUGR. The jeopardized fetus seems to be at the highest risk of imminent demise when Doppler abnormalities are observed in the arterial and especially venous circulation (ductus venosus and umbilical vein). The temporal sequence of abnormal Doppler changes in the peripheral and central circulatory systems of the growth restricted fetus have been described: early abnormal Doppler findings in the umbilical and middle cerebral arteries are followed by reversal of flow in the ductus venosus or pulsatile umbilical venous flow.

For this reason the use of Doppler velocimetry is of great importance in high-risk pregnancies and in cases of non-reassuring tests results.

9.3.3.1 Utero-Placental Vessels

Both the uterine and other utero-placental vessels have been studied with Doppler sonography.

Table 9.5 The most widely used indices used for studying utero-placental, placental and fetal arteries

Doppler indices	
Systolic/Diastolic ratio (S/D)	Systolic peak/end-diastolic velocity
Resistance Index (RI)	S-D/S
Pulsatility Index (PI)	S-D/mean velocity

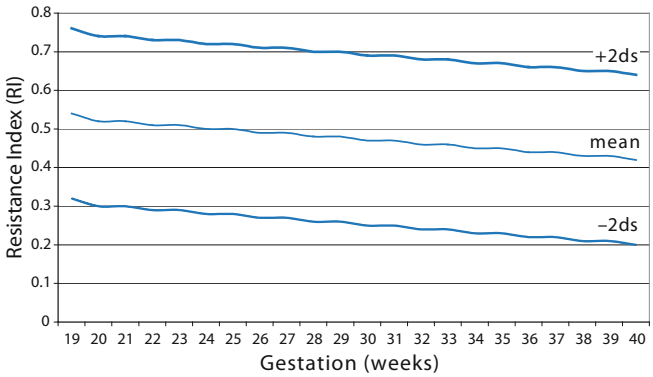

Fig. 9.2 Uterine artery resistance index

Uterine arteries are two symmetrical vessels that bring the oxygenated blood of the mother to the placenta.

There are numerous Doppler studies on uterine and spiral arterial flow during normal first trimester pregnancies which demonstrate a high vascular resistance that progressively disappears during the second trimester as a consequence of decreased resistance downstream. Decreasing vascular impedance with gestation (Fig. 9.2) is probably a consequence of dilatation of spiral arteries induced by trophoblast infiltration, hormonally mediated vasodilatation and a decrease in maternal blood flow viscosity. Thus, changes in uterine blood flow are correlated with the development of complications in later pregnancy: if the process of trophoblastic invasion of the spiral arteries' muscular walls is incomplete, the arteries are still able to respond to vasoactive stimuli, which results in vasoconstriction. In these pregnancies the uteroplacental circulation remains in a state of high resistance, which causes generalized endothelial cell injury, compromising vascular integrity and an atherosis-like process in the small arteries resulting in vessel occlusion, local ischemia and necrosis. As a consequence of vasoconstriction, a chronic inadequate blood supply to the growing placenta leads to a poor outcome (spontaneous abortion, IUGR, stillbirth, or preeclampsia).

When an abnormal adaptation of pregnancy occurs, impedance to flow increases and Doppler examination often demonstrates the presence of an high resistance index (RI) (the most significant quantitative parameter) and/or the presence of an early mono- or bi- lateral diastolic notch (qualitative parameter). The latter parameter is the abnormality correlated most closely with a poor pregnancy outcome.

Adverse outcomes associated with abnormal uterine artery flow velocity include: pre-eclampsia and fetal growth restriction and its sequelae, mainly fetal distress.

For this reason, in high-risk women, accurate utero-placental investigation during the first and second trimester of pregnancy allows the clinician to identify, and therefore to monitor, fetuses at high-risk of distress. Through the investigation of uterine artery flow in high-risk pregnancies, it has been shown that if the fetus has a normal umbilical flow, the compromise in growth will be mild, the risk of fetal distress slightly above normal, and the most common indication for intervention is maternal, not fetal. Present evidence does not support routine Doppler ultrasound for low-risk or unselected populations, which does not appear to benefit mother or baby [13].

Recent studies have shown that Doppler studies of the uterine arteries can be useful also during the third trimester of pregnancy: when both middle cerebral artery and uterine arteries show abnormal Doppler indices, the fetus has a significantly higher rate of poor outcome even in the presence of normal umbilical indices [14].

9.3.3.2 Feto-Placental Vessels

The umbilical artery was the first fetal vessel to be evaluated by Doppler velocimetry and Doppler velocimetry of the umbilical artery (UA) provides a non-invasive measure of the feto-placental hemodynamic state.

Umbilical arteries can be identified by Doppler ultrasound as early as 6–8 weeks, when the Doppler flow velocity profile shows only a systolic component. By 20 weeks, all fetuses should have end-diastolic flow in the umbilical vessels. As the pregnancy progresses, placental resistance shows a progressive decrease during gestation (Fig. 9.3) and there is an increasing diastolic flow velocity. A mature umbilical artery flow velocity waveform is usually achieved by 28–30 weeks, but some fetuses,, e.g. twins, may show delayed maturation. Several studies have revealed a large standard deviation up to 26–28 weeks in the umbilical S/D ratio that reflects variations in placental development. Eighty percent of the flow velocity waveform is a reflection of the resistance beyond the point of measurement, but 20% can be a forward flow effect that is reduced in cardiac output changes, arrhythmias, and bradycardia.

Abnormality of the pulsatility index (PI) (the most significant quantitative parameter) has been correlated with feto-placental vascular maldevelopment. Changes in umbilical artery velocity waveforms reflect changes in the placental bed vascular resistance. The primary and secondary branches of the umbilical circulation are in place after the first trimester.

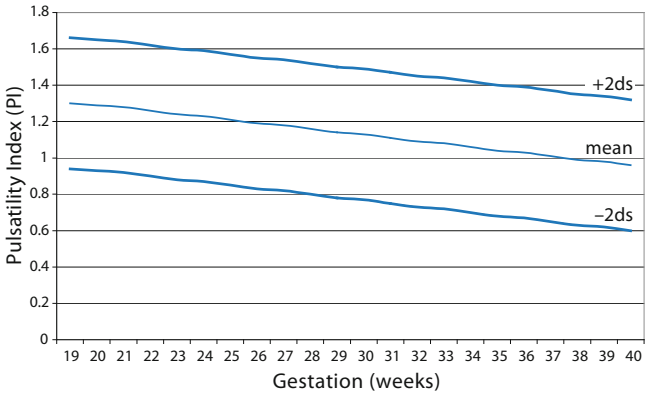

Fig. 9.3 Umbilical artery pulsatility index

For the next 3 months there is active proliferation of tertiary branches. Placental histology showed that growth-restricted fetuses with abnormal flow velocity have half as many tertiary arterioles as normal fetuses [15]. Pathological studies have demonstrated that increased impedance in the umbilical arteries becomes evident only when at least 60% of the placental vascular bed is obliterated.

Although it may not be a separated entity, absent or reversed diastolic (ARED) flow in the umbilical artery has been the subject of a considerable body of literature. Clinical studies of umbilical arterial flow velocity waveforms in IUGR have reported a progressive increase in impedance to flow until end-diastolic flow becomes absent and, in extreme cases, reversed. This is associated with a high perinatal morbidity and mortality. In pregnancies with ARED flow, the capillary loops in terminal villi are decreased in number, longer and with fewer branches than in normal pregnancies. For this reason, there is a strong association between the ARED velocity and hypoxia/acidosis [16].

When there is reversed flow, it may be a clinical emergency because most of these fetuses will die within 2 weeks. The ARED flow, as a test for hypoxia, shows a high sensitivity, specificity, positive predictive value and negative predictive value [17]. However, where flow is continuous throughout diastole, only a very high S/D ratio (greater than 4.5) is associated with hypoxia. Furthermore, the observation of ARED flow in the umbilical artery Doppler surveillance of pregnancies at risk for fetal distress has great clinical importance, especially in pregnancies associated with fetal growth restriction [18]. Umbilical artery Doppler applied to high-risk pregnancies significantly lowers perinatal mortality, the need for induction of delivery and the emergency cesarean section rate.

9.3.3.3 Fetal Vessels

Alteration in fetal vessel blood flow reflects an important hemodynamic modification that occurs in association with IUGR and fetal hypoxemia. The Doppler-detectable modifications in the fetal circulation associated with these conditions include increased resistance in both the umbilical artery (as described before) and fetal peripheral vessels, in association with decreased resistance in the fetal cerebral vessels.

Studies of other vascular beds in the fetus, including the cerebral circulation and the central arterial and venous systems, have increased the knowledge of compensatory and adaptive mechanisms and the time sequence of circulatory changes in the fetus subjected to hypoxia and severe growth restriction.

Arterial Compartment

The circle of Willis is an easy landmark to identify using ultrasound. Color flow makes it easy to see the common carotid and the anterior, middle and posterior cerebral arteries.

Measurement by Doppler of the peak systolic flow velocity (PSV) of the fetal middle cerebral artery (MCA) is a predictor of moderate or severe fetal anemia [19] and can be used to avoid unnecessary invasive procedures in pregnancies complicated by red blood cell isoimmunization.

Study of this vessel also yields important information for the detection of fetal distress. In some growth restricted fetuses, the cerebral vessels are dilated. Usually these are fetuses with reduced umbilical artery flow velocity. This implies that fetuses with mild hypoxia will have dilated cerebral vessels as a compensatory response: this explains the so-called "brain sparing" effect seen in asymmetrical growth restriction. These changes occur early and may be present for weeks or even months before birth. Some data suggest that they may persist into the early neonatal period. Study of the MCA is becoming essential for the understanding of fetal hypoxia, particularly in the early third trimester, when a surveillance program can be introduced (Fig. 9.4).

Fetal arterial Doppler studies are useful in the differential diagnosis of small for gestational age fetuses. In the hypoxemic group, due to impaired placental perfusion, the PI in the umbilical artery is increased and, in the fetal MCA the PI is decreased. Consequently, the ratio between the umbilical artery and middle cerebral artery PI (UA/MCA) is increased. An abnormally low UA/MCA ratio is associated with increased perinatal morbidity and mortality: when performed before 34 weeks of gestation. Use of the UA/MCA ratio seems to improve the prediction of perinatal outcome compared to the umbilical artery PI alone. However, the UA/MCA ratio does not appear to correlate significantly with fetal outcome when performed after 34 weeks' gestation. In third trimester fetuses, the ratio between PI in the fetal descending thoracic aorta and the MCA may be more useful.

In IUGR fetuses the trend changes of the MCA-PI and MCA-PSV provide more clinical information than one single measurement. A high MCA-PSV predicts fetal distress and perinatal mortality better than a low MCA-PI. For this reason MCA-PSV might be valuable in the clinical assessment of IUGR fetuses that have abnormal UA Doppler [20].

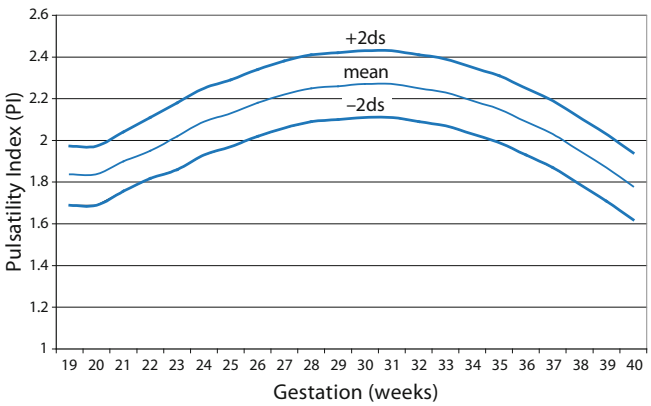

Fig. 9.4 Middle cerebral artery pulsatility index

Fetal Arterial Blood Flow Redistribution

The "brain-sparing" effect is a phenomenon where fetuses that are hypoxic preferentially perfuse the brain, heart, and adrenals at the expense of the integument, viscera, gut, and kidneys.

Although knowledge of the factors governing circulatory rearrangements and their mechanism of action is incomplete, it appears that partial pressures of oxygen and carbon dioxide play a role, presumably through their action on chemoreceptors. This mechanism allows preferential delivery of nutrients and oxygen to vital organs, thereby compensating for diminished placental resources. However, compensation through cerebral vasodilatation is limited and a plateau corresponding to a nadir of PI in cerebral vessels is reached at least 2 weeks before the onset of late fetal heart rate decelerations [21] and the development of the fetus is jeopardized. This suggests that maximal vascular adaptation to hypoxemia precedes the critical degree of impairment of fetal oxygenation, after which vascular dilatation may be suppressed by the development of cerebral edema. An alternative explanation may be that in severe hypoxemia, an increase in PI is the consequence of alterations in flow due to reduced cardiac contractility and to a fall in absolute cardiac output. Consequently, arterial vessels are not suitable for the longitudinal monitoring of growth-restricted fetuses and cardiac and venous velocity waveforms give more information about fetal well-being or compromise.

Cardiac and Venous Compartment

Cardiac flow is influenced by the modifications of arterial impedance to flow. As long as the fetus is able to compensate for a reduced placental supply by arterial redistribution (a brain sparing effect), there is preferential myocardial oxygenation, which delays the development of right heart failure despite an increasing afterload. Cerebral vasodilatation produces a decrease in left ventricular afterload, whereas increased placental and systemic resistance produce increased right ventricular afterload. At this stage, fetal Doppler measurements show high placental resistance and arterial redistribution in the presence of normal venous waveforms and the majority of fetuses have normal, reactive heart rate traces and biophysical profiles. Progressive changes in the venous circulation may indicate failure of the compensatory mechanism and herald the development of right heart failure due to myocardial hypoxia [22].

Longitudinal studies of deteriorating growth-restricted fetuses have shown that PSV and cardiac output gradually decline, suggesting a progressive worsening in cardiac function. Similarly, there is a symmetrical decrease in ventricular ejection force at the level of both ventricles, despite the dramatically different hemodynamic conditions present downstream of the two ventricles (i.e., reduced cerebral resistances for the left ventricle and increased splachnic and placental resistance for the right ventricle). This supports a pivotal role of the intrinsic myocardial function in the compensatory mechanism of the growth-restricted fetus following the establishment of the brain-sparing effect. Ventricular ejection force dramatically decreases in a short time interval (about 1 week), showing an impairment of ventricular force close to fetal distress. As a consequence, cardiac filling is also impaired [23].

Late Doppler changes generally accompany metabolic deterioration and are a result of declining cardiac function and abnormal organ autoregulation.

Increasing venous Doppler indices are the hallmark of advancing circulatory deterioration since they indicate a decreasing ability of the heart to accommodate venous return.

Fetal hypoxemia is associated with reduced umbilical venous blood flow, but, despite this decrease, a normal PSV in the ductus venosus is maintained. In the growth-restricted fetus, the percentage of umbilical venous blood passing through the ductus venosus is increased from about 40% (in normal fetuses) to about 60%. Thus there is a redistribution of venous blood flow in favor of the ductus venosus at the expense of hepatic blood flow in growth restricted fetuses. Unlike peak velocity during ventricular systole, flow velocities during atrial contraction are reduced or even reversed. One may speculate that increased end-diastolic right ventricular pressure would not influence ductus venosus blood flow velocities during atrial contraction, as flow is preferentially directed through the foramen ovale to the left atrium. However, the foramen ovale is closed during atrial contraction and blood flow velocity through the foramen ovale decreases to zero.

Altered venous flow velocity waveforms are more closely related to intrauterine fetal jeopardy than changes in arterial flow, which may occur quite early during the course of impaired placental function.

The degree of fetal acidemia can be estimated from Doppler measurements of pulsatility in both the arterial system and the ductus venosus. With moderate acidemia (pH between –2 and –4 standard deviations from the normal mean for gestational age), almost all fetuses have a MCA PI below 2 standard deviations, whereas there is a wide scatter of individual results for the ductus venosus, with the majority of measurements being still within the reference ranges. With increasing severity of hypoxemia and acidemia, ductus venosus PI increases [24] and, in the most severe cases, velocities with atrial contraction were reduced to zero or even become negative.

However in severe fetal growth restriction, abnormal Doppler velocimetry of the ductus venosus is the only significant parameter associated with perinatal death and low 5 minutes Apgar scores [25].

Doppler Findings and the Prediction of Perinatal Risks

Elevations of placental blood flow resistance and venous Doppler indices frequently progress in parallel. The recognition

of venous Doppler changes has led to re-examination of the relationship between prenatal Doppler findings in growth restricted fetuses and perinatal outcome.

Previously, delivery was recommended when umbilical artery Doppler end-diastolic velocities were absent or reversed. However, the Growth Restriction Intervention Trial (GRIT) [26] showed that if there was early onset IUGR, early and preterm delivery were independent risk factors for adverse neurodevelopment at 2 years of age. Safe prolongation of pregnancy in these fetuses is therefore critical. Daily biophysical profile scoring in fetuses with absent or reversed umbilical artery end-diastolic velocity with strict delivery criteria has been associated with good outcomes, suggesting that safe prolongation of these pregnancies is indeed possible. It is now apparent that prediction of perinatal risk based on umbilical artery alone, without accounting for venous circulatory changes, is inadequate. Prediction of stillbirth and acidemia are significantly enhanced by the addition of venous Doppler findings, irrespective of the umbilical arterial end-diastolic velocity. This rate increases when the arterial end-diastolic velocity is absent or reversed but venous Doppler indices are still normal, and increases further when venous Doppler indices become abnormal (predominantly due to an increase in the rate of stillbirth). If the prevalence of stillbirth is 25%, abnormal venous Doppler findings have 65% sensitivity and 95% specificity for the prediction of stillbirth. Depending on the cutoff (2 *vs* 3 SD) and the combination of veins examined, the sensitivity for acidemia ranges from 70% to 90% and specificity from 70% to 80%. Randomized trials investigating the utility of venous Doppler in the management of preterm growth restricted fetuses are underway.

9.4 During Labor Surveillance

9.4.1 Identification of Fetal Distress

Maternal blood flow to the placenta may be severely diminished by uterine contractions, so the potential for fetal hypoxia increases during labor. In the last century some asphyxiated fetuses were noted to have abnormally fast or slow heart rates, so heart auscultation evolved as a component of intrapartum care. However, the human ear is insensitive to subtle changes in rate, so electronic methods of recording have been developed. These methods generate paper traces that show features not obvious on auscultation, including the degree of variation of the heart rate and the shape of accelerations and decelerations in rate (the CTG).

Identification of fetal distress based on fetal heart rate patterns is imprecise and intrapartum Doppler velocimetry was studied as a potential adjunct to conventional intrapartum fetal monitoring but it was concluded that this technique was a poor predictor of adverse perinatal outcomes [1].

9.4.2 Monitoring of Fetal Heart Rate

The pattern of the fetal heartbeat during labor is often a good indicator of fetal well-being. The normal fetal heart rate (FHR) is 120–160 bpm. A normal heart rate suggests that the fetus is receiving enough oxygen from the mother's bloodstream. The typical fetal heart rate pattern is to slow somewhat during a contraction and increase again at the end of a contraction. Abnormal variations in heart rate can indicate decreased oxygen in the blood and tissues of the fetus that can potentially damage the fetus. The fetal heart can be monitored either intermittently by auscultation or continuously by CTG.

9.4.2.1 Auscultation with Fetal Stethoscope or Doppler Ultrasound Stethoscope

The fetal heartbeat and variability can be checked before, during, and after contractions, either using a Pinard fetal stethoscope or a Doppler ultrasound stethoscope, a small hand-held device which uses sound waves to monitor the heart beat. In low risk births, the American College of Obstetricians and Gynecologists [27] suggests monitoring at least every 30 minutes during active labor, and at least every 15 minutes during the second stage of labor. When risk factors are present, the fetal heart should be evaluated every 15 minutes, and every 5 minutes in the second stage. The mother's abdomen may be also palpated to check for the strength of contractions.

9.4.2.2 Electronic External or Internal Monitoring

In 2002, approximately 3.4 million fetuses (85% of approximately 4 million live births) in the United States were assessed by continuous cardiotocography, also known as electronic fetal monitoring (EFM), making it the most commonly performed obstetric procedure.

A hand-held Doppler ultrasound probe can be used to calculate and display, but not permanently record, the FHR. Electronic fetal monitors are used to both determine the FHR and continuously record it in graphical form. External measurement means taping or strapping the electrodes to the maternal abdominal wall, with a small Doppler ultrasound heart electrode overlying the fetal heart, and the contraction electrode measuring the tension of the abdominal wall, an indirect measure of the intrauterine pressure. Both electrodes are connected to video screens and a printer which produces graphs of the fetal heart rate and "intensity" of the contractions.

Internal measurement requires the cervix to be at least 2 cm dilated as it involves inserting a pressure catheter into the uterine cavity, as well as attaching a scalp electrode to the fetal head to adequately measure the heart rate. Membranes must be ruptured to place the electrodes. The electrode measures the fetal heart rate. It is used in some high-risk births if

the external monitor shows suspicious results or if external monitoring is not generating an adequate trace. Internal monitoring offers better accuracy of readings than external monitoring, but there is an increased risk of infection after application of the electrode. The advantage of internal monitoring is that it is immediately apparent if the fetal heart rate slows down, stops, or is slow to recover from contractions. It can detect reduced oxygen flow due to cord compression (more common after rupture of the membranes), oxytocin augmented contractions, or narcotic and epidural related changes in maternal blood pressure.

During the *first stage* of labor, a moderate fetal tachycardia may be considered a circulatory mechanism of adaptation against moderate fetal hypoxia. By contrast, a severe tachycardia is a clear sign of fetal hypoxia: it can be associated with high maternal temperature, or fetal cardiac malformations or medications administered to the mother. The same is true for a bradycardia: a moderate bradycardia is considered without clinical significance, but a marked bradycardia is a sign of severe fetal hypoxia.

Similar considerations relating to the use of CTG apply as during the antepartum period for what concerns *baseline variability*. Accelerations are, as before labor, an expression of normality. Early decelerations are not usually a sign of fetal distress. They are likely generated by vagal stimulation due to cephalic compression and are more frequent after rupture of the membranes.

Variable decelerations are usually due to umbilical cord compression. They may occur in association with fetal distress due to hemodynamic changes. However, late decelerations are a typical expression of fetal hypoxia. Their timing and shape are directly correlated with the severity of fetal hypoxia: the time elapsing between the contraction and the deceleration is directly proportional to the fetal partial oxygen pressure.

Obviously, in the presence of both late decelerations and baseline abnormalities the prognosis is more serious.

Interpretation of the cardiotocogram is more difficult during the *second stage* of labor. In this period only a severe and persistent bradycardia is considered of serious prognotic significance, especially when associated with abnormalities in variability.

If the CTG is normal during labour, intermittent auscultation may be appropriate. It is considered normal if the FHR is between 120 and 160 bpm. It is also suggested that monitoring should be undertaken for 20–30 minutes every 2 hours during labor.

The ability of the EFM to identify the fetus that may be becoming asphyxiated and may therefore benefit from intervention is limited, and its use has failed to lead to reduced rates of cerebral palsy and neurologic injury [28]. There are no studies comparing CTG with an absence of intrapartum monitoring, but trials comparing CTG with an intermittent auscultation showed no reduction in the overall risk of perinatal death (relative risk [RR] 0.85; 95% confidence interval [95% CI] 0.59–1.23) or cerebral palsy (RR 1.74; 95% CI, 0.97–3.11) [29]. What studies have demonstrated is that CTG compared with intermittent auscultation leads to the over-diagnosis of fetal distress: the "false positive" rate for fetal distress diagnosis is estimated at between 40% and 71% [30] and also leads to higher operative delivery rates by cesarean section or assisted vaginal delivery (RR 1.66; 95% CI; 1.30–2.13 and RR 1.16; 95% CI, 1.01–1.32, respectively). For this reason, Continuous Electronic Fetal Monitoring should be reserved for high-risk pregnancies (Table 9.6).

Table 9.6 High-risk pregnancies

- Abnormalities on intermittent auscultation or at CTG monitoring at the beginning of the labor
- Discolored amniotic fluid
- Oxytocin infusion
- Fever during labor
- Other conditions of pathological labor

If CTG monitoring shows a non-reassuring FHR many sources recommend fetal scalp sampling to determine whether there is fetal distress and whether interventions are necessary (see below).

There is controversy regarding electronic monitoring in high-risk situations, such as premature labor, placenta or cord problems, or when there is a complicating disease like diabetes, hypertension, or sickle cell anemia. There is little evidence that EFM is beneficial compared to frequent auscultation.

However, as the Cochrane review [29] concluded with some dejection, there is little evidence that the use of EFM will diminish in the near future. It seems that we will have to wait for the advent of new technologies, which will be more effective in monitoring fetal well-being during labor.

9.4.3 Monitoring of Fetal Oxygenation

9.4.3.1 Pulse Oximetry

Fetal pulse oximetry has been advocated as a means of improving the specificity of CTG in intrapartum fetal surveillance.

The first-line method for evaluating fetal oxygenation during labor is electronic fetal heart rate monitoring. Despite the extensive use of FHR monitoring during labor, it has a poor specificity of 38%, and its sensitivity is 94% for detecting fetal death and hypoxia [31]. As explained before, a high proportion of FHR tracings are not reassuring, even when the fetus is in good condition. Until recently, the only adjuvant for the assessment of fetal hypoxia and acidosis was fetal scalp blood sampling, which is invasive and traumatic to the fetus and can only assess the fetus on an intermittent basis and can be inaccurate if contaminated with amniotic fluid. Fetal pulse oximetry was introduced into clinical practice

about 30 years ago, but it has only recently been possible to use it for the management of fetal well-being. It was developed as a less traumatic and invasive method of assessing fetal oxygenation than fetal scalp sampling. It allows for the real-time and continuous assessment of the fetus [32, 33]. It uses a probe that sits inside the vagina during labor. It is possible only if the membranes are ruptured because the probe has to be placed on fetal skin.

Fetal oxygen saturation decreases between the first and the second stages of labor (from 60% to 53%): a value of 30% is considered pathological. A fetal oxygen saturation of 30% for 10 minutes is associated with acidosis and a poor fetal outcome. Low values of fetal oxygen saturation are associated with an abnormal CTG trace (low variability) [34].

There are still controversies about the benefits of fetal pulse oximetry. Some authors have concluded that fetal pulse oximetry during active labor provides a more accurate assessment of fetal well-being and may reduce the need for interventions. In the view of others, the addition of fetal pulse oximetry does not reduce overall cesarean section rates. A Cochrane review comparing EFM alone with EFM plus fetal pulse oximetry included one randomized control study (RCT) involving 1,010 participants. There was a reduction in the rate of cesarean deliveries performed for non-reassuring tracings, but no difference in overall cesarean section (CS) rates [35]. Since then, three additional RCTs have similarly failed to show benefit [36–38]. A better method for the evaluation of fetal well-being in labour is required [39]. For this reason, the use of this device is not recommended because there is insufficient evidence of any benefit. Its use is not endorsed by the American College of Obstetricians and Gynecologists.

9.4.3.2 Fetal Scalp Blood Sampling

A sample of blood from the fetal scalp may be tested during labor to determine the acidity of the blood. This test is called fetal blood sampling. If the fetus is not getting enough oxygen, the blood becomes highly acidic.

Fetal blood sampling is typically performed using a kit. An amnioscope with a light source is used to expose the fetal scalp. The blood is collected in long, heparinized capillary tubes. The test requires that the cervix be dilated at least 2–3 cm, and can be difficult to perform.

Capillary blood collected from the fetal scalp usually has a pH lower than umbilical venous blood, and correlates well with fetal arterial values. However, scalp edema can result in erroneous results. A scalp pH value of less than 7.20 has traditionally been used to represent the critical value for identifying fetal acidosis (Table 9.7). However, a scalp pH below 7.15 is more representative of the tenth percentile and is closer to the threshold currently used in umbilical cord blood analysis to define fetal acidemia associated with neurological deficits.

Table 9.7 Recommended fetal management according to the pH value obtained by fetal scalp blood sampling

pH value	Management
pH >7.25	Repeat test if CTG continues to deteriorate
pH 7.21–7.24	Repeat test in 30 minutes
pH <7.20	Aim for delivery within 30 minutes

The accuracy of intermittent fetal scalp pH assessment for predicting neonatal acidosis with subsequent neurologic sequelae has been questioned. It is no longer used in many institutions, although its use can result in fewer cesarean deliveries performed for the indication of non-reassuring fetal status.

The degree of technical skill required, cost, need for continuous availability of standardized equipment and trained personnel and maternal discomfort have precluded use of this modality in many labor and delivery units.

The test has poor sensitivity and positive predictive value (PPV) for predicting umbilical arterial pH <7.0 (sensitivity 35%, PPV 9%). The test also has poor sensitivity and PPV for identifying newborns with hypoxic-ischemic encephalopathy (sensitivity 50%, PPV 3%) [40].

9.4.3.3 Fetal Electrocardiography

A technical system, the fetal heart monitor, monitors the fetal electrocardiogram (ECG) during labor. It can be used as an adjunct to continuous electronic FHR monitoring during labor. Use of this device is based on the principle that fetal hypoxemia can result in elevation or depression of the ST segment. The monitor's software automatically identifies and analyzes changes in the T wave and the ST segment of the fetal ECG, which is obtained via a spiral electrode attached to the fetal scalp. The use of ST waveform analysis has been associated with statistically significant reductions in the number of scalp sampling procedures. A Cochrane review showed no difference in perinatal death, neonatal encephalopathy, cerebral palsy, low Apgar scores, neonates with severe metabolic acidosis at birth, neonatal intensive care unit admission or cesarean delivery rate between controls (EFM alone) and study subjects (EFM plus STAN) [41, 42]. Thus, this technique appears to improve the clinician's ability to discriminate between fetuses in need of intervention and those who can be managed expectantly.

The fetal heart monitor has been approved by the United States Food and Drug Administration as an adjunct to the assessment of non-reassuring FHR tracings in pregnancies over 36 weeks of gestation, in labor, with vertex presentation and ruptured fetal membranes.

The device should not be used if there is a contraindication to placement of a fetal scalp electrode. This technique is promising, but at this time there are inadequate clinical and cost data from a variety of hospital settings to allow a recommendation for routine use.

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10

Multiple Pregnancies

Maria Angela Rustico, Mariano Lanna and Enrico Ferrazzi

10.1 Epidemiology of Natural Twinning

10.1.1 Actual Trend

The cultural modifications in women's reproductive strategies over the past 50 years have drastically influenced the demographic scenario. Higher levels of education and a desire for economic and social success, as well as difficulties in finding a permanent job, have played a major role in modifying the reproductive strategies, as evidenced by delayed parenthood and the reduction in family size [1]. In addition to this expected twinning rate due to advancing maternal age, there is also evidence that natural twinning has increased in women who have been exposed to assisted reproduction technologies [2].

10.1.2 Dizygotic Twinning

In humans, multiple pregnancies arise more frequently from fertilization of two separate oocytes (1.2% of pregnancies). The incidence of excessive follicular recruitment is more or less 31% in mothers of dizygotic (DZ) twins. The DZ twinning rate is affected by race, genetic factors, seasonality, maternal age and parity, endogenous gonadotropins, fertility drugs and sex of the embryo [3]. The incidence of twin pregnancy also varies from country to country, the highest rates being reported in Nigeria (5%), and the lowest in Japan. In Italy the best estimate of DZ twinning is 1%. It has been suggested that genetic predisposition and malnutrition may play a role in this different frequency. Maternal age up to 35–39 years is linked with increasing twinning rates and this pattern has been attributed to the rise in the level of gonadotropins with age [2]. There is a slight tendency for twins to have been conceived during summer as compared with singletons; and this may reflect the effect of increased light during summer on the pineal gland, resulting in melatonin production, and consequent decreased inhibition of pituitary follicle-stimulating hormone (FSH) release [3].

10.1.3 Monozygotic Twinning

The constant frequency of monozygotic (MZ) pregnancies over time and in different areas, and the lack of association between maternal age and frequency of MZ twinning, suggests that MZ twins are largely determined by genetic mechanisms. In fact an excess of monozygotic twins occurs in mothers who are themselves one of monozygotic twins, whereas there is no evidence for a paternal effect on MZ twinning [2].

10.2 Epidemiology of Iatrogenic Twinning

There have always been some naturally, or spontaneously, occurring multiple pregnancies (with twins being more frequent than triplets, and quadruplets or even higher multiples being much rarer). However, the frequency has increased enormously since assisted reproductive technologies (ART) have become available. Because of the higher morbidity and mortality associated with twins and particularly triplets, every effort should be made to reduce incidence [3]. In Europe, the overall rate of multiple pregnancy in 2002 was 23% compared to 26% in 2000. This reduction has been determined by the transfer of fewer embryos, except in Italy where a controversial law obliged the transfer of three embryos until it was recently modified. A similar high prevalence of twinning in ART pregnancies was reported in 2006 in the USA (26%), where such a policy had not been universally adopted. In the

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

USA the proportion of multiple births attributable to ovulation induction or ART is 33%. In triplets and higher-order multiple pregnancies this rate must be even higher.

10.3 Placentation

10.3.1 Chorionicity and Zygosity

Two thirds of spontaneous twin pregnancies are dyzygotic (DZ), resulting from the fertilization of two eggs by different spermatozoa, and are therefore dichorionic (DC). The DC twin placenta of DZ twins may be composed of separate disks, or alternatively, the two placental portions may be fused. Vascular communications are almost totally absent. The other third of spontaneous twin pregnancies are monozygotic (MZ), occurring when one single fertilized egg gives rise to two separate embryos. Of these MZ pregnancies, a third are DC. The other two thirds are monochorionic diamniotic (MCDA), meaning that a single placenta serves both embryos, and that vascular communications connecting the twins are always present. Between 2 and 5% of monochorionic (MC) twins are monoamniotic (MCMA). When twinning occurs after approximately 12 days from conception Siamese twins are generated (Fig. 10.1).

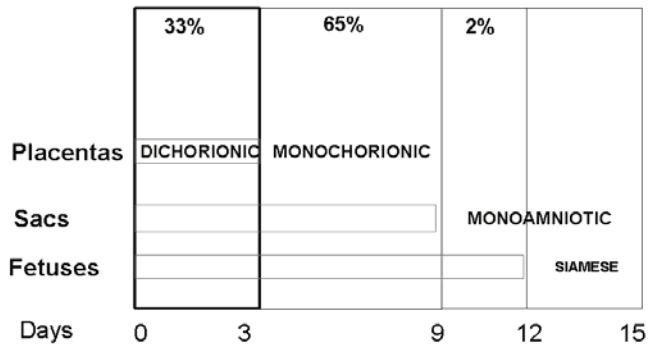


Fig. 10.1 Partition of placentas and chorion, amniotic sac and fetuses as a function from days of conception in monozygotic twinning

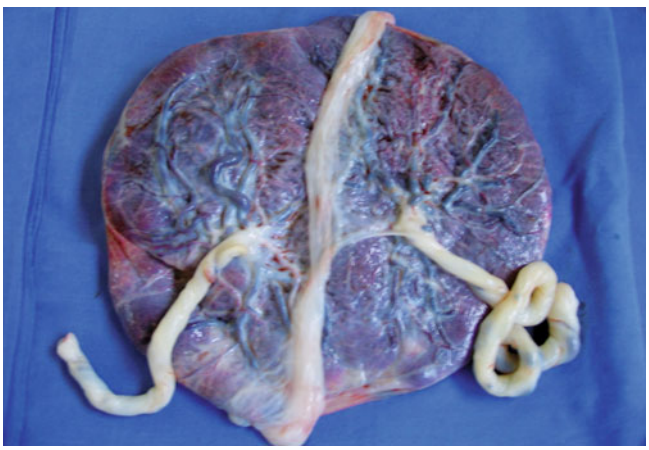


Fig. 10.2 Monochorionic placenta

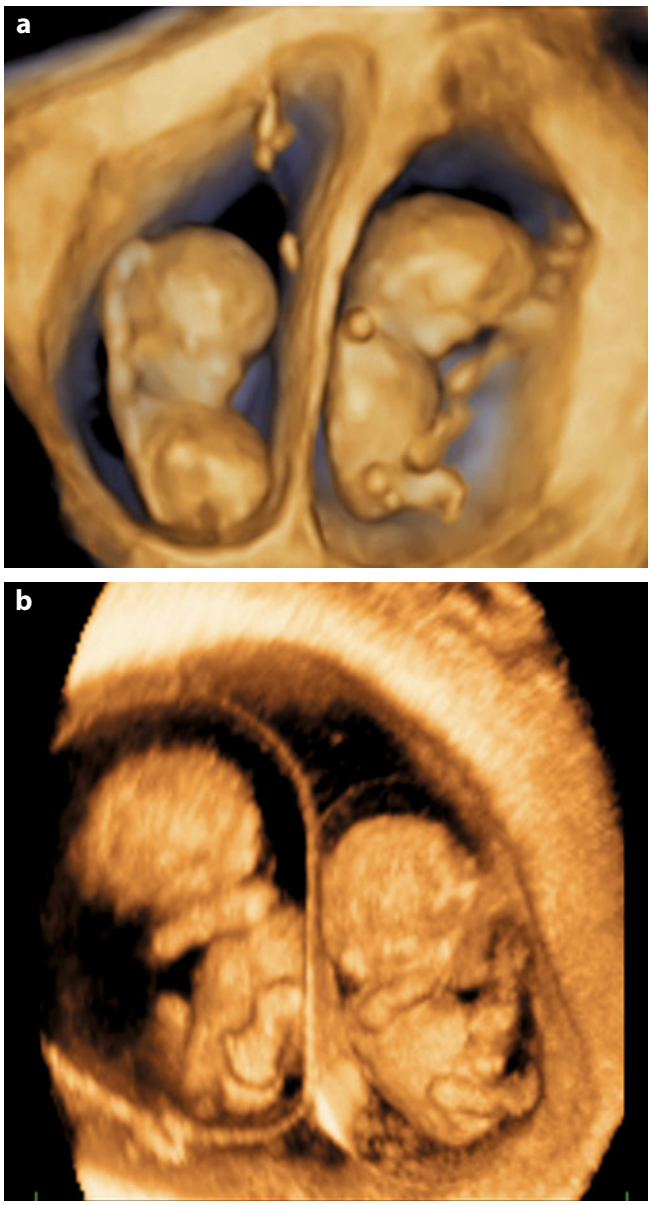


Fig. 10.3 **a** Dichorionic twins (lambda sign): chorion and decidua between the two sacs. **b** Monochorionic twins (T sign), only extraembryonic coelom between the two amniotic membranes

Because of their placental angioarchitecture (Fig. 10.2), MC twins are more susceptible to additional complications than DC twins. In fact, the presence of vascular anastomosis, which mediates unidirectional or bidirectional flow between the two circulations, greatly influences intrauterine development and plays a crucial role in causing those complications specific to MC twins, leading to a higher morbidity and mortality than in DC twins [4].

In multiple pregnancies, ultrasound is a very accurate technique (100% sensitivity and 99% specificity) for determining chorionicity (Fig. 10.3). In twin pregnancies, in the first or early second trimester, ultrasound examination is mandatory to determine chorionicity in order to assess the obstetric risk which is significantly different in DC and MC twins.

10.4 Fetal Complications of Twin Pregnancy

10.4.1 Miscarriage and Fetal Loss

10.4.1.1 Definitions

The World Health Organization in 2008 reported a consensus on definitions concerning miscarriage and fetal loss. Preclinical spontaneous miscarriage: a pregnancy diagnosed only by the detection of HCG in serum or urine, which does not develop into a clinical pregnancy. Spontaneous abortion/miscarriage: the spontaneous loss of a clinical pregnancy as diagnosed by ultrasound that occurs before 20 completed weeks of gestational age (18 weeks post fertilization) or, if gestational age is unknown, the loss of an embryo/fetus of less than 400 grams. Vanishing sac(s) or embryo(s): spontaneous disappearance of one or more gestational sacs or embryos in an ongoing pregnancy, documented by ultrasound.

10.4.1.2 Epidemiology

The "vanishing twin" phenomenon, which has been viewed as part of the general fetal wastage in human reproduction, suggests that while multiple conceptions are not rare events, multiple pregnancies are strongly selected against, especially during the early stages of embryonic development. The incidence of miscarriage during the first trimester is estimated to be between 10 and 20%. The true incidence of vanishing twins is difficult to assess. Landy and Keith [5] reviewed the majority of pertinent studies, most of which described pregnancies conceived as a result of ART. Using these data it was estimated that ~30% of these twins will ultimately result in singletons and <10% will end in a complete abortion.

10.4.1.3 Etiology

It is thought that intrinsic abnormalities within the embryo are the major reasons for failed conceptions or early fetal death. The most significant intrinsic factor contributing to embryonic loss is aberrations in the first meiotic division resulting in non-dysjunction and aneuploidy. Trisomies 13, 15, 16, 18 and 21 account for the most common autosomal trisomies in spontaneous pregnancy losses. Some authors have observed an increased risk of fetal death, and in particular spontaneous abortion with increasing maternal age, and there is clear evidence from oocyte donation programmes that this risk is associated with the ageing of the oocytes [6].

10.4.1.4 Diagnosis

The diagnosis of miscarriage is made by ultrasound (US) examination. A biochemical pregnancy is considered when implantation is revealed on day 16 by blood test, but there is a negative US scan result on day 23 [7].

10.4.2 Fetal Growth Restriction

10.4.2.1 Definition

Intrauterine growth restriction (IUGR) is a sonographic diagnosis consisting of either an estimated fetal weight below the 5th percentile of the intrauterine growth chart for gestational age, or declining abdominal fetal growth below the 5th percentile, or declining more than 40 centiles from mid-pregnancy to third trimester. Abnormal uterine Doppler velocimetry and/or an abnormal umbilical Doppler velocimetry are considered additional criteria of severity (Italian Society of Ultrasound in Obstetrics and Gynecology). In a twin pregnancy, a growth discordance of more than 20% in estimate fetal weight is an index of selective growth restriction.

10.4.2.2 Epidemiology

Neonates from multiple gestations are over-represented among preterm and low birth weight infants. Multiple pregnancies weigh less than their singleton counterparts. The prevalence of small for gestational age newborns in multiple pregnancy is higher than expected in singletons. Although many variables may influence the reference standard, the prevalence of small for gestational fetuses varies between 12 and 47%.

10.4.2.3 Etiology

For most of a pregnancy, twins grow at the same rate as singletons, regardless of chorionicity, up to at least 32 weeks' gestation. Thereafter, twins show a slower rate of growth [8]. The decreased rate may be related to uteroplacental insufficiency. It is thought that, at some point in the third trimester, the placenta can no longer maintain the nutrient requirement of both fetuses. In patients with triplets or high-order multiples, this process occurs earlier.

When growth restricted fetuses are diagnosed in a twin pregnancy, one should keep in mind the diverse etiologies of IUGR (such as genetic/chromosomal problems, fetal anatomical anomalies, placental and cord abnormalities, maternal

complications) rather than assuming that the cause is uteroplacental insufficiency, which is more frequent in twins than singletons. Although IUGR can complicate a pregnancy with growth discordance, the latter does not necessarily imply the former unless growth discordance is less than 20%. It is important to note that IUGR can affect both twins, leading to both twins being small but not discordant

10.4.2.4 Diagnosis

Knowledge of chorionicity assessed during the first trimester or by gender discordance is paramount for managing patients with growth abnormalities. Since fetal growth is a dynamic process, serial ultrasound is helpful in the assessment of fetal growth in patients with multiple fetuses. In utero growth discordance is most often defined as the difference in sonographic estimated fetal weights expressed as a percentage of the larger twin's estimated fetal weight. Growth discrepancy may be mild (<15%), moderate (15–30%), or severe (>30%). This wide range (from 15 to 30%) is the consequence of clinical neonatal examination. It might help to note that a study by Naeye [9] in 1964 showed that the total number of organs and cells is not reduced until growth discrepancy is more than 25%. However, in less severe cases, weight discrepancy is determined by a smaller volume of cell cytoplasm. A clinical diagnosis of growth discordance is better achieved by adding information about feto-placental conditions to biometric growth curves: for example, amniotic fluid index, Doppler examination of the umbilical artery and fetal vessels, computerized analysis of fetal heart rate, maternal complications that frequently cause placental insufficiency such as gestational hypertension and preeclampsia.

10.4.2.5 Consequences

IUGR has long been known to be associated with perinatal morbidity and mortality. Neonatal morbidity such as meconium aspiration syndrome, hypoglycemia, polycythemia and pulmonary hemorrhage may affect up to 50% of IUGR neonates. The high prevalence of IUGR in twin pregnancies suggests that such patients should be included in protocols relating to the management of high risk pregnancies.

10.5 Preterm Delivery

10.5.1 Definition

Preterm birth (PTB) is defined as a delivery at less than 37 completed weeks' gestation. This condition can be further divided into late preterm (33–37 weeks), moderate preterm (28–32 weeks), and severe preterm (20–27 weeks).

10.5.2 Epidemiology

There is a wide variation in preterm birth rates between countries, mostly because of different iatrogenic practices. In many states of the European Union, about half the children born following multiple births were preterm, accounting for between 18 and 25% of preterm birth in each country. The proportion of births before 37 weeks in twins ranges from 68.4% in Austria to 42.2% in the Republic of Ireland. This reflects different clinical protocols and the consideration of twin pregnancies being at high risk and providing parents with leave from work and other social maternal benefits.

10.5.3 Etiology

The etiology of preterm birth depends on multiple factors. The most investigated etiologies both in singletons and twins are infection and maternal stress. Twin pregnancy with its impact on cervical integrity is per se a risk factor for ascending infections. Twin pregnancy is also a risk factor for psychological and physical maternal stress. Other minor risk factors for preterm birth are: cervical incontinence, uterine malformations, infertility, previous preterm birth or intrauterine fetal death, low social status, male fetal sex.

10.5.4 Diagnosis

High risk for PTB should induce clinicians to make a careful assessment of risk factors and to use appropriate diagnostic tests. Among these the evaluation of short cervical length by transvaginal sonography seems to be the best predictor of PTB. Infection and other possible direct causes of PTB should be excluded. Fetal fibronectin in vaginal secretions may add to the usefulness of cervical length measurements. A cervical length ≤25 mm at 18 weeks and ≤22 mm at 24 weeks seems the best predictors of preterm delivery.

10.5.5 Prevention

Cervical length measurement, the fibronectin test and the assessment of uterine contractions might diagnose impending PTB, but should not be confused with the cause of PTB. Efforts should be made to exclude intrauterine infections and subclinical chorioamnionitis. Blood and amniotic tests for infections and abnormalities of fetal heart rate should be take into account. Tocolysis with Atosiban and bed rest may delay delivery by at least 48 hours, permitting the administration of corticosteroids to improve fetal lung maturity. In cases of severe infection, delivery may well be the best option combined with antibiotic therapy. Opinions about cervical cerclage vary.

10.5.6 Fetal Complications

Prematurity is the main cause of low birth weight, perinatal mortality, and the most frequent determinant of neonatal and infant mortality and morbidity. The following diseases are frequently associated with prematurity: transient tachypnea of the newborn, respiratory distress syndrome, persistent pulmonary hypertension, respiratory failure, temperature instability, jaundice, feeding difficulties, intraventricular hemorrhage, necrotizing enterocolitis and brain damage.

10.6 Monochorionic-Related Complications

10.6.1 Twin-to-twin Transfusion Syndrome

Twin-to-twin transfusion syndrome (TTTS) is the best-known complication of MC pregnancies, occurring in approximately 10–15% of cases. Although hormonal and hemodynamic mechanisms may be involved, the current interpretation of TTTS is that it is caused by an imbalance in the exchange of blood between one twin (the donor), and the other (the recipient) via placental anastomoses, due to a relative excess of unidirectional arterio-venous connections which is not compensated by reverse flow through other anastomoses. When a significant imbalance in blood flow occurs, the donor twin becomes hypovolemic and oliguric, develops severe oligohydramnios and Doppler signs of placental insufficiency. The recipient twin shows hypervolemia, polyuria and polyhydramnios, with cardiac overload, leading to hydrops in severe cases [10]. Without treatment, the prognosis is poor, with perinatal mortality up to 90%. In survivors, preterm birth due to polyhydramnios is a major cause of mortality and morbidity. In the case of intrauterine death of one twin, the surviving twin has a substantial risk of neurologic morbidity due to hemorrhage into the dead co-twin via placental anastomoses [11]. At 4 years follow-up, the incidence of cerebral palsy and abnormal mental development is 21% in surviving twins with TTTS [12].

The traditional neonatal criteria for diagnosing TTTS, based on an inter-twin hemoglobin difference (> 5 g/100 mL) and weight discordance (> 20%), do not apply in utero because similar discrepancies in hemoglobin and birthweight are also found in DC and MC twins without TTTS [13].

The diagnosis of TTTS is only made by ultrasound. In a twin pregnancy with a single placental mass, twins of the same gender, and a thin membrane dividing the twins (Fig. 10.3), the crucial ultrasound sign for the diagnosis of TTTS is the combined presence of polyuric polyhydramnios in one sac (deepest vertical pocket > 8 cm before 20 weeks' gestation, >10 cm after 20 weeks' gestation), and oligouric oligohydramnios in the other (deepest vertical pocket <2 cm) [14].

A classification system is divided into five stages based on ultrasound criteria. Stage I or II is when the donor bladder is still either visible or empty, in association with the polyhydramnios-oligohydramnios sequence. Stage III is when Doppler findings are abnormal for either twin. Stage IV and V is when there is congestive cardiac failure and hydrops in the recipient or the death of one or both twins [15]. This classification has created an impression of a disease with progressive deterioration and poorer outcome in the more advanced stages. However, clinical observations have demonstrated that the natural history of TTTS is variable and unpredictable. For this reason, this classification has recently been challenged because it fails to incorporate the cardiac function of the recipient twin, in whom there may be myocardial dysfunction even in Stage I and II in over 50% of cases, which represent advanced TTTS [16].

TTTS can manifest itself at any time during a pregnancy, but is more common in the second trimester. Considering the poor survival rate, and the risk of neurological complications due to antenatal or postnatal injury, treatment should undoubtedly be offered. The Eurofetus randomized trial which compared laser coagulation of placental anastomoses with serial amnioreduction, demonstrated that laser coagulation is the best first-line treatment for TTTS diagnosed before 26 weeks because it blocks the vascular connections which are assumed to be responsible for the syndrome [14].

10.7 Single Intrauterine Death

In MC twin pregnancies, the death of one twin, occurring in about 4% of these pregnancies, presents the co-twin with an increased risk of mortality and morbidity. The mechanism for this adverse outcome is blood loss from the dying twin through placental vascular anastomoses. This leads to hypovolemia, which may cause the death of the co-twin from hypovolemic shock, or parenchymal damage due to hypoperfusion. Sequelae include ischemic cerebral lesions, periventricular leukomalacia, renal cortical necrosis, and small bowel atresia. A review of the literature of 119 MC twin pregnancies complicated by the death of one of the fetuses showed 57% healthy survivors, 19% perinatal deaths, and 24% survivors with severe sequelae [11]. The risk of an adverse outcome for the surviving co-twin depends on the gestation at the time of intrauterine death, and the interval to delivery. In early pregnancy, the death of one twin frequently causes the death of the other fetus, but severe sequelae in the survivor are less common than at a more advanced gestation. The same review showed that in healthy survivors there was a longer interval to delivery (mean, 11.1 weeks) when compared to pregnancies with perinatal deaths or neurologic sequelae (mean, 5.3 weeks). Immediate delivery after a single intrauterine death only adds the risks of prematurity to the surviving twin. Indeed, damage may occur at the moment of death of the co-twin and therefore may not be preventable. Intrauterine transfusion can be a therapeutic solution but, if the critical moment preceding fetal death is missed, there

is no treatment and conservative management is recommended [17]. Ultrasound of the fetal brain and magnetic resonance imaging (MRI) 2–3 weeks after an intrauterine fetal death may provide useful information for predicting neurodevelopmental outcome in the surviving twin.

10.8 Twins Discordant for Fetal Anomalies

An excess of structural anomalies is observed in twins compared to singletons. While the frequency of malformations in a DZ pregnancy is similar to that for singletons (2–3%), it is two or three times higher than in a MZ pregnancy [18]. The reason for this is unknown, but might be considered to be part of teratogenic process during twinning.

MZ twins are considered identical, but recent observations have reported that this is not the rule, and a number of discordances have been observed for chromosomal anomalies (such as trisomy 21 and Turner syndrome), single gene disorders, X-linked diseases (such as Fragile-X, Aicardi's syndrome) and structural defects. These structural defects include malformations of the brain, abdominal wall anomalies, and cardiac disorders. The prevalence of cardiac defects has been reported as 2.3% in twins without TTTS, and 7% in those with TTTS, compared to 1% in the general population [19].

Structural malformations which are not genetically determined may of course affect only one of the two MZ twins. It has been estimated that both twins are affected in less than 20% of cases, whilst in the majority of cases only one twin is involved.

In approximately 1–2% of twin pregnancies a serious anomaly affects only one fetus and clinicians face the dilemma of choosing between expectant management or selective termination. The primary goal is to prevent the death of the normal twin. In monochorionic twin pregnancies, selective termination needs to be performed by ensuring complete and permanent occlusion of umbilical cord of the abnormal twin, in order to avoid acute hemorrhage from the co-twin into the dying fetus, a process which may lead to death or organ damage [20]. Bipolar cord occlusion under ultrasound guidance is still considered the best method for selective feticide in these cases.

Table 10.1 Type of discordant anomalies detected in a series of 84 monochorionic twins

Type of discordant anomaly	N. (%)
Central nervous system	37 (44.0)
Cardiovascular (without TTTS)	12 (14.3)
Abdominal wall	8 (9.5)
Urinary tract	8 (9.5)
Skeleton	3 (3.6)
Miscellaneous	10 (11.9)

Based on a cohort of 84 (9.6%) discordant anomalies detected by ultrasound or invasive prenatal diagnosis in 870 monochorionic twin pregnancies between 1999 and 2009, Table 10.1 shows the type of severe anomaly detected in one fetus that led to selective termination by bipolar cord coagulation in 51.2% of cases. During the same period, five twin pregnancies were complicated by concordant anomalies (trisomy 21, Apert's syndrome, cystic hygroma with hydrops, bilateral renal dysplasia) affecting both fetuses (0.6 %).

10.9 Twin Reversed Arterial Perfusion (TRAP) Syndrome

Twin reversed arterial perfusion (TRAP) sequence, also known as acardiac twinning, is a rare complication of MC pregnancies which can lead to an unfavorable outcome for the normal co-twin. The reported incidence is 1/35,000 deliveries, or 1% of MZ twins. In this condition there is a twin without any functional cardiac tissue and variable developmental disruption (the acardiac twin). This acardiac twin is perfused by the normal twin (the pump twin) by arterial blood flowing in a retrograde fashion through a single superficial artery-to-artery placental anastomosis. Poorly oxygenated blood bypasses the placenta, enters the acardiac twin's circulation at low pressure, and preferentially perfuses its lower part. The blood flow returns to the pump twin via a single vein-to-vein connection [4]. The acardiac twin acts as a parasite and space-occupying mass, being hemodynamically dependent on the other twin. Perinatal mortality rates reported for the pump twin range from 35–55%.

The primary causes leading to poor perinatal outcome are preterm delivery (because of the continuing growth of the acardiac fetus) and congestive heart failure (because of the volume of the acardiac twin's mass needing to be perfused by the normal heart). The deoxygenated blood circulating back to the pump may also cause chronic hypoxia, growth restriction, and hypoxic ischemic lesions.

In the case of rapid enlargement of the acardiac twin mass, or signs of cardiac overload in the pump twin, blockade of the vascular supply to the parasitic twin is recommended. Possible treatments include intrafetal ablation procedures such as radiofrequency and interstitial laser. Although no single technique has been conclusively shown to be the best, some form of antenatal treatment seems to be beneficial, because there is a reported overall pump twin survival rate of 76%.

In a series of 34 cases of TRAP sequence during the period 1999–2009, 15 cases were managed expectantly. In six cases, intrauterine death of both twins occurred before 18 weeks, while one pregnancy was terminated because of an abnormal pump twin. In eight cases, both twins were delivered near term. Two babies (where the acardiac fetus was very large, up to 1.5 kg) had a poor neurological outcome and periventricular leukomalacia probably because of the pathophysiology of the

condition. Apart from complications related to the invasive procedures (4/19), and one case of unexplained intrauterine late death of the pump twin, 14 of 19 cases (73.6%) who had in utero treatment are alive and well and free of neurological complications at postnatal follow-up.

10.10 Monoamniotic Twinning

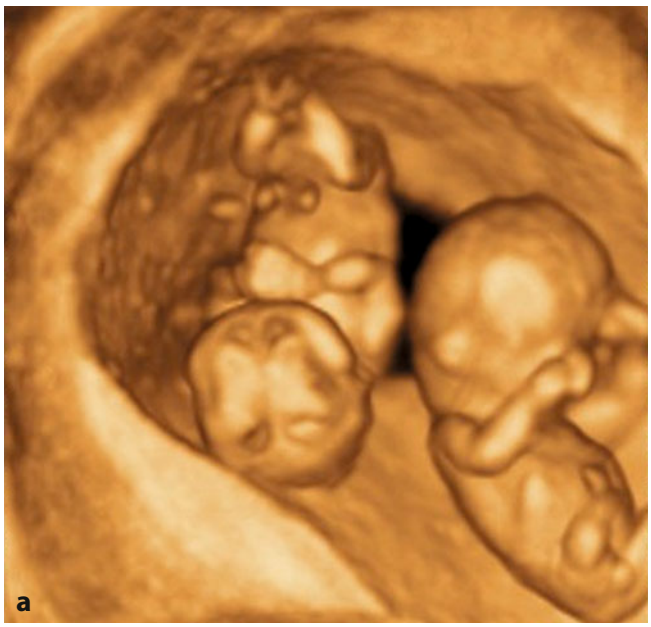
Monoamniotic (MA) twinning is rare, affecting 2% of all MZ pregnancies. MA twins share the placenta and the amniotic sac, and are at higher risk of structural anomalies, unexpected fetal death and perinatal death (ranging from 30 to 70%), compared with MCDA twins. The excess of fetal loss can be explained by umbilical cord entanglement (Fig. 10.4), and a crucial cofactor may be an acute exsanguination across large placental anastomoses. In the largest study reported in the literature (98 cases) there was a 17% perinatal mortality rate, and fetal death after 32 weeks occurred in 4% of pregnancies [21]. Thus the current perinatal mortality rate in MA twins appears to be lower than previously reported, but it remains high and occurs throughout pregnancy. Based on the observation that the risk of neonatal death associated with preterm birth at 32 weeks is 1 in 100, the risk for MA twins at this gestational age appears to be four times lower.

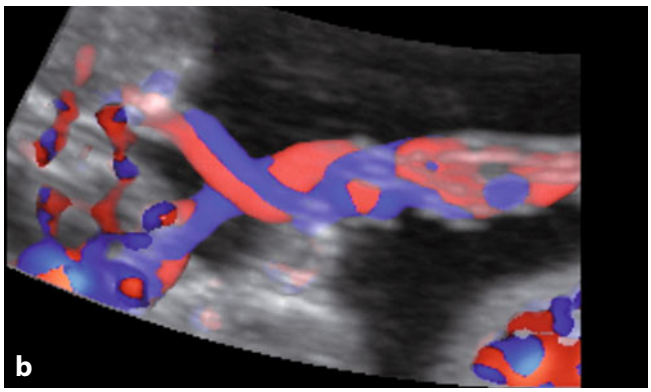
10.11 Laser Treatment In Twin-to-twin Transfusion Syndrome

Since the description of vascular anastomoses affecting all monochorionic twin placentae, the development of TTTS syndrome was explained by the hypothesis of unbalanced flow through arterio-venous anastomoses. Thus, the aim of researchers has been to treat TTTS by endoscopic laser coagulation of chorionic vessels in order to interrupt unwanted vascular connections. In the first description, De Lia photocoagulated all possible anastomotic vessels [22].

Due to a lack of anatomical markers for the identification of communicating vessels, this technique has been difficult to reproduce, and other approaches have been proposed. The non-selective technique requires coagulation of all vessels crossing the insertion of the inter-twin membrane. The selective technique interrupts only those vessels involved in blood exchange between twins, preserves normal cotyledons and is considered an important development in the treatment of TTTS [23].

The procedure is performed under local or regional maternal anesthesia. A 3 mm cannula is introduced in the amniotic sac of the recipient twin under ultrasound guidance using a percutaneous approach. A 2 mm fiberscope is then passed through the cannula, so that the operator can explore the fetal surface of the placenta to identify the vascular connections.





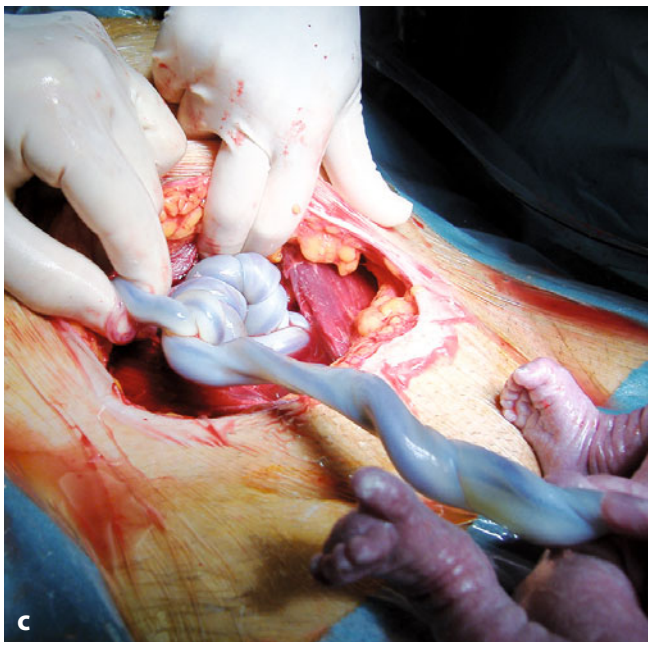


Fig. 10.4 Monochorionic monoamniotic pregnancy: twins floating in the same sac (**a**); entanglement of umbilical cords with colour Doppler (**b**); true knots between the two cords (**c**)

After identifying the anastomosis between an artery and a vein arising from the donor twin, coagulation is performed with a 400 μm laser fibre.

Until recently, serial amnioreduction (AR) was the most common treatment for TTTS. Amnioreduction works by reducing the risk of preterm delivery secondary to uterine distension, and possibly also by reopening compensatory placental vascular anastomosis. For MC twin pregnancies complicated by TTTS before 28 weeks, the International Amnioreduction Registry reported an overall survival rate at birth of 78%. In survivors, a central nervous system scan was abnormal in 25% of cases at 1 month of life [24].

In 2004, endoscopic laser coagulation of chorionic vessels was shown to result in a better neurological outcome than serial amniotic reduction. Periventricular leukomalacia (PVL) was 6% *vs* 14% (p = 0.002) [25]. This is why laser treatment is considered the best first line option in severe TTTS before 26 weeks' gestation, with 6 months' survival ranging from 50 to 70%. Complications that lead to a repeated procedure (laser or amniotic reduction are reported in less than 5% of cases, and reflect the evidence that there are deeper anastomoses, which cannot be coagulated. Preterm premature rupture of membranes (pPROM) has a significant impact on pregnancy when presenting before 24 weeks, since it may lead to miscarriage (7–23% of treated pregnancies) [26].

Perinatal survival rates vary from 50% in the first reported series to 80% in the most recently reported series (Table 10.2).

Postnatal complications have been described both in ex-recipient and in ex-donor twin after laser therapy. The recipient twin, which is hypervolemic, may develop right heart hypertrophy with a tricuspid valve regurgitation leading to valvular pulmonary stenosis. This cardiac abnormality may persist after successful laser treatment because myocardial tissue may have been already damaged by persistent overload. In selected cases, pulmonary stenosis may be so severe that balloon valvoplasty is required after birth. Similarly, severe hypovolemia may cause renal insufficiency. In this case, laser treatment can preserve kidney function in the surviving twin by establishing a normal circulating volume.

For both twins, neonatal morbidity is mainly related to neurological outcome. Most cerebral lesions result from hemorrhagic or ischemic injury. These lesions, which affect 10% of survivor twins after laser, are related to unbalanced blood flow and occur before laser treatment of TTTS [27]. Cerebral palsy affects nearly 6% of twins after laser; the risk of neurodevelopment impairment increases significantly with

Table 10.2 Endoscopic non selective laser coagulation of placental vessels: pregnancy outcome

Authors	N. cases	GA (weeks) procedure	GA (weeks) delivery	Overall survival rate (%)	Live fetuses after procedure (%)			
					2	1	≥1	0
De Lia et al, 1995 [22]	26	20.8 (18–24)	–	–	35	35	70	30
De Lia et al, 1999 [28]	67	21 (18–24)	–	69	57	18	75	25
Senat et al, 2004* [14]	72	20.6 (15–26)	33.3 (26–35)	57	36	40	76	24
Yamamoto et al, 2005 [29]	175	20 (16–24)	–	54	35	38	73	27
Robyr et al, 2006** [30]	101	21	32.1	76	66	22	88	12
Quintero et al, 2000 [23]	71	20.8 (16–25)	32.7 (24–39)	61	39	43	83	17
Chang et al, 2006 [31]	428	20.1 (16–26)	32	80	66	29	95	5
Hecher et al, 1999 [32]	73	20.7 (17–25)	33.7 (25–40)	61	42	37	79	21
Hecher et al, 2000 [33]	127	20.7 (16–26)	34 (23–40)	68	54	27	81	19
Huber et al, 2006 [34]	200	20.7 (16–25)	34.3 (23–40)	71	59	24	83	17
Becker et al, 2006 [35]	31	19.7 (16–26)	32.3 (26–37)	66	–	–	81	19
Barrea et al, 2006 [36]	35	21	30	66	43	46	89	11
Buzzi Hospital (2004–2009)	155	20.6 (16–27)	31.6 (23–40)	52	33	39	72	29

GA gestational age.
* Randomized multicenter study ** Multicenter study.

Table 10.3 Cord occlusion in complicated monochorionic twin pregnancies: pregnancy outcome *

Indication	N. cases	GA (wks) at procedure median (range)	IUD n (%)	TOP n (%)	Ab n (%)	pPROM <24 wks n (%)	PD <32 wks n (%)	GA (wks) at delivery median (range)	PND n (%)	Overall surv n (%)
TTTS	45	22.3 (17.1–27)	5 (11)	1 (2)	4 (9)	4 (9)	13 (29)	33 (25–40)	5 (11)	30 (67)
sIUGR	30	21.6 (16–26.6)	2 (7)	0	0	2 (7)	9 (30)	33.5 (23.5–40)	2 (7)	26 (87)
Anomaly	38	22.1 (17–25)	5 (13)	0	3 (8)	3 (8)	8 (21)	37 (25–41)	4 (10)	26 (68)
Total	113	22.1 (16–27)	12 (11)	1 (1)	7 (6)	9 (8)	30 (26)	34 (23.5–41)	11 (10)	82 (73)

TTTS twin-to-twin transfusion syndrome, *sIUGR* selective intrauterine growth restriction of one twin, *GA* gestational age, *IUD* intrauterine death, *TOP* termination of pregnancy, *Ab* abortion, *PD* premature delivery, *pPROM* premature rupture of membrane, *PND* perinatal death.
* Fetal Medicine Unit, Buzzi Children's Hospital, Milan (1999–2009). Unpublished data.

prematurity. Neurological outcome worsens when laser treatment is performed at advanced gestational age and with a III/IV Quintero stage of TTTS. The reason is that the fetal brain may be more vulnerable to injury when the gestational age at the time of the procedure is higher. Indeed, the rate of neurological lesions in surviving twins after the intrauterine death (IUD) of one twin is considerably lower after laser therapy than after spontaneous IUD.

10.12 Cord Occlusion in Monochorionic Twins

Bidirectional flow through vascular anastomoses makes blood pressure in each twin dependent on both circulations: after the spontaneous IUD of one twin, acute hypotension in the other twin causes death or neurological damage in 50% of cases [11]. In complicated MC pregnancies, the risk of IUD of one twin is so high that selective termination of pregnancy (TOP) can be the last opportunity to save at least one fetus. There is often severe intrauterine growth restriction (IUGR) of one twin with an abnormal umbilical cord Doppler signal and TTTS that persists after treatment (amniotic reduction or laser), or there may be discordant abnormalities.

Intracardiac injection of chloride potassium cannot be used, as it would in dichorionic twin pregnancies because the toxic drug could reach the other fetus through anastomoses causing an unwanted fetal death. The safest way to terminate one MC fetus is occlusion of the umbilical cord of the selected twin. This can be done by ligation or by bipolar forceps coagulation, procedures that also prevent unwanted hypotension and hemodynamic changes in the co-twin. Bipolar cord coagulation appears to be a better option because of a higher overall survival rate [37]. With a local anesthesia, a 3.3 mm cannula is introduced in the uterine cavity under ultrasound guidance. Coagulation is obtained by a power of 50 W for 30–40 sec with the aim to reduce blood flow in the placenta of the selected fetus.

In a cohort of 113 pregnancies that underwent umbilical cord occlusion with bipolar coagulation, preterm delivery occurred in 33% of cases, with a procedure-to-delivery interval which varied between 1 to 22 weeks (median 11 weeks) (Table 10.3).

As for any other invasive procedure, pPROM before 24 weeks is the main complication (8%), and it is associated with abortion in 85% of cases if the procedure is performed before 18 weeks. A recent review reported a prevalence of 4–7% of severe neurological lesions in surviving MC twins [38]. This percentage is lower than expected if compared with neurological morbidity in surviving twins after the spontaneous IUD of the co-twin, which accounts for 22% of cases.

The main problems associated with any invasive intrauterine therapeutic procedures are abortion and prematurity following pPROM. The possibility of sealing the membrane damage caused by trocars and needles has recently been investigated by the introduction of an amniopatch made of platelets and cryoprecipitate. It seems that, if injected into the amniotic cavity, an amniopatch is able to seal ruptured membranes in 50–70% of spontaneous pPROM [39]. This level of success is not reached in cases of iatrogenic rupture of the membranes, where the origin of damage depends on many confounding factors (i.e., indication for the procedure, experience of the operator, gestational age at the time of the procedure).

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11

Intrauterine Growth Restriction: Obstetric Aspects

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11.1 Etiology of Intrauterine Growth Restriction

Intrauterine growth restriction (IUGR) is not a specific condition, but represents the epiphenomenon of a variety of conditions. Traditionally, it is etiologically associated with maternal, fetal and placental disorders (Table 11.1). However, it is useful to make some distinctions within the group of IUGR pregnancies.

First, a clear definition of IUGR is needed. From a statistical viewpoint, the term "small for gestational age" (SGA) has been used to define newborns whose birth weight falls below the 10th percentile of gestational age specific birth weight standards [1]. This retrospective definition has been extremely useful in the past to provide a better understanding of perinatal outcomes. However, the widespread use of ultrasound in obstetrics has changed the traditional way of looking at fetal growth during pregnancy and specific intrauterine growth charts have been developed [2]. From an obstetrician's viewpoint, today the term IUGR indicates deviation from the intrauterine growth trajectory, based on intrauterine growth charts derived from formulae which take into account biometrical parameters measured in utero [2, 3]. Considerable differences have been reported between intrauterine and birth weight curves, especially at the lowest gestational ages [4, 5].

Although premature babies are often defined as low birth weight (LBW), very low birth weigth (VLBW) or extremely low birth rate (ELBW), IUGR is often a significant feature for these babies [6]. However it is important to distinguish babies who are premature because of spontaneous preterm delivery (most of these neonates are appropriate for gestational age) from babies who are premature because of iatrogenic preterm delivery, since the majority of the most severe IUGR infants will be delivered prematurely for clinical indications (Table 11.1). Thus, IUGR represents a risk factor for prematurity and not the opposite. Similarly, in most studies relating maternal malnutrition to IUGR, it is really an association with SGA that is reported [7].

A second important point is that the traditional list of risk factors for IUGR is misleading in separating mother, fetus and placenta as independent determinants of IUGR. Many risk factors share common factors and a distinction between placental and nonplacental causes of IUGR would be more appropriate.

A third point is that it is now widely recognized that severe IUGR is associated with arterial and/or venous Doppler abnormalities [8].

11.1.1 Placental Causes of IUGR

The placenta should always be analyzed when an IUGR fetus is born as there are few cases of IUGR where the placenta is macroscopically and/or microscopically normal.

Placental weight is often reduced, depending on the severity of the disease [5, 9, 10] and the reduction in placental mass is then inadequate to meet fetal needs throughout pregnancy. However, even when placental weight is normal and the fetus is IUGR, it is likely that the functional capacity of the placenta (measured as placental function per gram of tissue) may be impaired.

Several placental abnormalities have been reported in IUGR placentas [9]. Those associated with hypertension, either chronic, pregnancy induced or preeclampsia, are probably the most studied. A recent hypothesis suggests that there are two categories, placental and maternal [11]. Placental preeclampsia comprises placental vascular abnormalities, immunological and placental oxidative stress, which determine the maternal disease and growth restriction. In the maternal form, which often presents late, the placenta is normal (as is the growth of the fetus), but the maternal response is abnormal.

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 11.1 Risk factors associated with intrauterine growth restriction

Maternal factors
Maternal diseases
– Hypertension/Preeclampsia
– Chronic hypertension
– Renal disease
– Pregestational diabetes
– Autoimmune syndromes (antiphospholipid, lupus erythematosus)
– Thrombophilia
– Cyanotic heart disease
– Asthma
– Hemoglobinopathy
– Phenylketonuria
– Uterine anomalies
– Therapeutic agents
Maternal life style
– Smoking
– Substance abuse (alcohol, drugs)
– Low socio economic status
– Short interpregnancy interval
– Infertility/ART
Environment
– Malnutrition
– Pollution
– Living at high altitude
– Living in developing countries
Fetal factors
Congenital anomalies
– Genetic diseases (aneuploidy, uniparental disomy, etc)
– Malformations
– Infections
Preterm birth
Multiple gestation
Unexplained elevated alpha-fetoprotein
Placental factors
Confined placental mosaic
Placenta previa
Abruption placentae
Infarction
Circumvallate placenta
Placenta accreta
Hemangioma
Cord abnormalities

Modified from [25].

Placental abnormalities have been demonstrated in cases involving maternal diabetes (hypervascularity of the villi/choriangiosis), maternal hypertensive disorders, maternal substance abuse (villus ischemic change/villus infarction) and maternal thrombophilia (villus ischemic change/infarction/thrombi). Abnormalities with SGA and LBW have also been reported [12].

Isolated abnormalities of the placenta and the umbilical cord can result in IUGR. Marginal/membranous insertion or hypercoiling of the umbilical cord have been found to be associated with vascular abnormalities [10], which may interfere with umbilical blood flow. The most common abnormalities seen in the placenta of IUGR are maternal vascular obstruction, fetal vascular obstruction, villitis of unknown etiology (either focal or patchy/diffuse), perivillous fibrin(oid) deposition and chronic abruption.

In summary, knowledge of the risk factors which have been reported to be associated with IUGR may impact considerably on clinical management both prospectively (when the risk factor is present and known at the beginning of the pregnancy and determines appropriate treatment) and retrospectively (for the thorough investigation of possible causes once an ultrasound diagnosis of intrauterine growth failure has been made). Histological examination of the placenta is mandatory whenever an IUGR baby is born (as with any obstetric adverse outcome) since it may reveal the presence of an underlying disease process which may have an impact on the long-term outcome of mother and child. However, prospective studies relating IUGR and its severity (as indicated by Doppler abnormalities) are needed to define the risk factors for this pathology, as most studies have been retrospective and related to neonatal birth weight.

11.2 Intrauterine Growth Restriction

Battaglia and Lubchenco [1] proposed that babies below the 10th percentile when born be considered SGA. These population data were based on birthweights of infants born at each gestational age. However, preterm delivery itself represents significant obstetric pathology. Later, as ultrasound was improved, biometry by ultrasound was found to be more reliable.

Studies have addressed which aspect of fetal body measurements provides the best estimate of fetal weight. These data led to the construction of fetal growth curves [2], which had the advantage that measurements could be made on normal pregnancies throughout gestation, including the 3 or 4 months before term delivery. Hence data from normal pregnancies were used to construct the fetal growth curves for early fetal development. This approach resulted in the calculation of the rate of fetal growth (from the slope of the estimated weight curve), instead of having to rely on a single body weight measurement obtained after birth.

The use of fetal growth data based on ultrasound measurements was a major advance in diagnosing and tracking fetal growth restriction. However, it still relied on population data to construct "norms" and, as many investigators pointed out, such an approach can lead to the misdiagnosis of "normal small" fetuses born to small parents. Gardosi [4] introduced standards to correct the fetal growth curve for parental size. Using this approach, the maternal size was the dominant determinant. While this was an improvement, it has largely been displaced by an approach that combines utilization of

biometric data obtained by ultrasound with velocimetry data obtained by power Doppler analysis [13]. Infants that are small by fetal growth data and have changes in the umbilical circulation indicating increased vascular resistance are clearly IUGR. This approach has several distinct advantages. Firstly, the two measurements, (ultrasound biometry and umbilical artery velocimetry) are both fairly routine in high risk obstetrical services. Secondly, the approach combines an assessment of fetal size with a functional assessment of the fetal circulation. This approach eliminates the misdiagnosis of small babies who are small for genetic or other reasons, and who have no obstetric pathology.

The disadvantage of this approach is that it is possible to miss babies whose fetal growth is restricted by placental or other pathology, but who have not yet developed the circulatory changes associated with increased vascular resistance in the umbilical circulation. This need not be a major clinical problem if reassessment of the umbilical circulation is repeated later during pregnancy.

Hence, at this time, the recommended approach is one which limits the diagnosis of IUGR to infants who have both reduced fetal size and elevated umbilical arterial pulsatility indices with the stipulation that the evaluation of umbilical arterial velocimetry be repeated several times during pregnancy.

11.2.1 Evaluation of Fetal Well-being

There have been major advances in monitoring the health of the fetus as pregnancy progresses. The most striking have been in the area of the evaluation of the circulation [14]. We will discuss this from a physiological basis, where velocimetry data are obtained from different fetal vessels. This approach is most widely used since power Doppler techniques permit the rapid collection of such information from different vessels. These approaches will be discussed first. However, it should be emphasized that absolute blood flow requires not only measurement of red cell velocity, but also measurement of the vessel's diameter.

11.2.2 Velocimetry Data

Studies began with measurements of umbilical artery velocimetry. In pregnancies complicated by IUGR, the umbilical arterial velocimetry pattern was different to that seen in uncomplicated normal pregnancies. They are characterized by a higher systolic peak and lower diastolic velocities, usually expressed as the Pulsatility Index (PI). The changes have been well described in many publications [15]. In the most severe cases, there is either no diastolic flow or reversed diastolic flow. The changes have been interpreted as indicating increased impedance in the umbilical circulation.

Since the umbilical circulation represents a large proportion of the fetal cardiac output, other studies followed, reporting changes in other part of the fetal circulation. Of special interest to neonatologists are the following changes which occur in some, but not all, IUGR pregnancies [16].

The fetal middle cerebral artery may show decreased pulsatility index, indicating vasodilatation. This has been interpreted as a physiologic response by the fetal cerebral circulation to a decrease in fetal oxygen tension.

As the condition of the fetus deteriorates in severe IUGR, the peak aortic and pulmonary artery flows may fall, indicating decreasing cardiac output. There are also changes in the ductus venosus with dilatation of the inlet of the vessel leading to flow velocity changes in the vessel [15, 16]. This will be discussed further under blood flow changes.

Pardi et al [13] proposed a classification of clinical severity of IUGR pregnancies which utilized fetal heart rate monitoring (FHR) and umbilical artery velocimetry data. This was a first attempt to define IUGR pregnancies according to the clinical impact on the fetus. The Pardi study showed that classification into three subgroups correlated with the incidence of fetal acidosis and hypoxia. Subsequent studies established that other metabolic parameters correlated with this rather crude clinical classification. For example, the transplacental glucose gradient, which is increased in IUGR pregnancies, was highest in Group 3 pregnancies and lowest in Group 1 pregnancies [17]. Because of the widespread use of FHR and umbilical artery velocimetry, this classification has continued to be useful.

More recently, Ferrazzi et al [15] examined the velocimetry changes at different points in the fetal circulation of IUGR pregnancies. Their work, as well as that of others, suggested that there is an orderly sequence of circulatory changes in IUGR pregnancies as physiologic conditions deteriorate with advancing pregnancy [15]. Specifically, the studies suggest that there are velocimetry changes which occur "early" in IUGR pregnancies; these include umbilical artery increased PI, dilatation of the middle cerebral artery, and absent umbilical artery diastolic flow.

Table 11.2 and Fig. 11.1 present a summary of these findings. It is likely that future studies of IUGR pregnancies will utilize such information to better determine clinical severity in these pregnancies than just the umbilical artery PI and FHR.

Table 11.2 Classification of clinical severity in IUGR

Early findings	Late findings
UA PI	DV S/a
MCA PI	UA RDF
UA AEDF	DV RF
	AO, PV ↓

UA umbilical artery, *MCA* middle cerebral artery, *DV* ductus venosus, *AO* aorta, *PI* pulsatility index, *AEDF* absent end diastolic flow, *RDF* reverse diastolic flow, *RF* reverse flow, *PV* peak velocity.

11.2.3 The Advent of Blood Flow Measurements

It has always been known to physiologists that the only important determinant from the viewpoint of tissue perfusion and oxygenation was not the speed of blood flow (velocimetry measurements) but actual (volume) blood flow. Such measurements became feasible through Doppler techniques which measured the speed of the blood and the diameter of the vessel. From these two measurements, the blood flow in the vessel can be calculated. This information is extremely important in IUGR pregnancies, for the following reasons.

It should be emphasized that blood flow alone, that is the flow in mL min^{-1}, cannot be properly interpreted without some reference point. The usual reference is per kg of tissue perfused, although an even better reference might be per unit O_2 consumption. The first measurements as they relate to perinatal medicine were of umbilical blood flow. Because of the size of the umbilical vein and the fact that it has little in the way of vessel pulsations, this measurement can be made very accurately in human pregnancies and these data have been reported from many centers [18, 19]. IUGR pregnancies have a striking reduction in umbilical blood flow, not only in absolute terms, but also when expressed per kg fetal weight [20]. Thus, in terms of fetal oxygenation, the IUGR fetus operates with a much smaller margin of safety and anything that might further adversely affect flow could lead to fetal hypoxia.

The reduced umbilical flow has another significance for neonatologists. A large fraction, (~60%) of the umbilical blood flow perfuses the fetal liver and the remainder is shunted through the ductus venosus into the inferior vena cava. In more severe IUGR pregnancies, the ductus venosus shunt can greatly increase [18]. The result of the combined effects of a reduced umbilical blood flow [21] and a larger shunt of this reduced flow through the ductus leaves the fetal liver with very little blood flow. This may explain neonatal cases of hepatocellular damage in IUGR infants without evidence of infection.

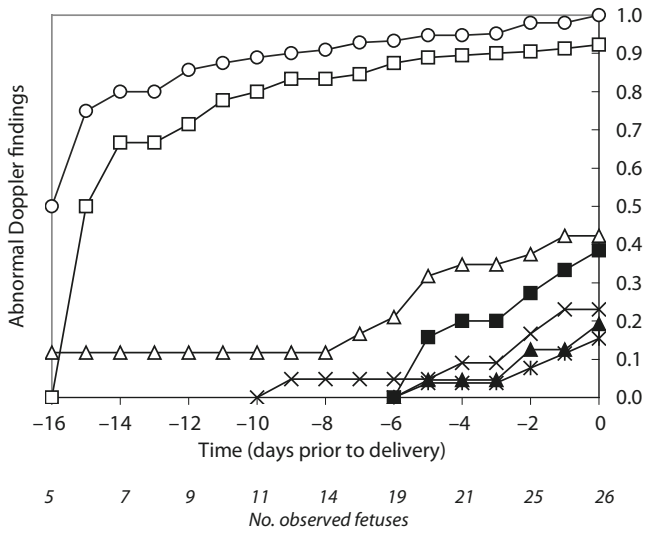

Fig. 11.1 Cumulative onset time curves of Doppler abnormalities for each fetal vessel examined. Time '0' refers to the date of delivery. ○ MCA PI; □ UA AEDF; △ DV S/a; ■ UA RF; × PA PV; ▲ DV RF; ✳ AO PV

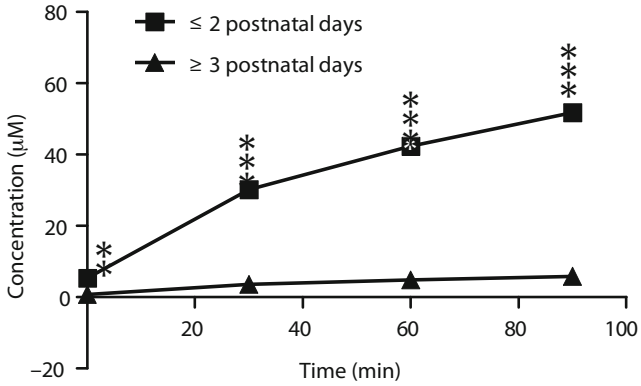

Fig. 11.2 Postprandial galactose concentrations are significantly higher after a milk feed in infants 2 days or less after birth than older infants. Data from [24]

It is now well established that the ductus venosus remains patent in many healthy infants born at term [22, 23] and IUGR infants who may have developed hypoxia and acidosis in utero may be prone to a persistent ductus venosus for a longer postnatal period of time. As a consequence of both the fetal hepatic injury from hypoperfusion and the persistent ductus venosus, galactose concentrations can be markedly elevated following a milk feed (Fig. 11.2) [24].

11.2.4 Fetal Hypoxia

It has been known for many years that pregnancies complicated by IUGR have a relatively high incidence of fetal hypoxia. This was also evident in the study of Pardi et al [13] where Group 3 pregnancies had an incidence of hypoxia and acidosis of approximately 70% compared to 0% in Group 1 pregnancies without velocimetry changes. From our previous discussion, it is clear that the high risk group could be further narrowed down to a group with "late" velocimetry changes. Persistent fetal hypoxia may also explain the increased incidence of neonatal polycythemia and hyperviscosity. The acidosis of clinical significance is metabolic. CO_2 crosses the placenta in both directions very rapidly. Transient changes in perfusion of the uterine and/or umbilical circulations at the time of delivery can lead to a very high PCO_2 in the fetus but as soon as neonatal ventilation is established, the PCO_2 is rapidly blown off and the pH corrected.

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12

Intrauterine Growth Restriction: Neonatal Aspects

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12.1 Definitions

Although the terms *small for gestational age* and *intrauterine growth restriction* are often used as synonyms, they reflect two different concepts.

Small for gestational age (SGA) refers to a statistical definition, based on auxological cross-sectional measurements (prenatal or neonatal), and denotes a fetus or neonate whose anthropometric variables (usually weight) are lower than a given threshold value computed on a set of infants of the same gestational age. SGA includes infants who have not achieved their own growth potential because of maternal, uterine, placental and fetal factors as well as small but otherwise healthy infants [1].

Intrauterine growth restriction (IUGR) refers to a clinical and functional condition and denotes fetuses unable to achieve their own growth potential: a fetus with IUGR would have been larger, had no adverse environmental or genetic factors affected growth. IUGR can be recognized by ultrasonography during pregnancy by a longitudinal evaluation of fetal growth.

A neonate identified as SGA by neonatal anthropometric charts is not necessarily a case of IUGR and, conversely, a neonate identified as having IUGR during the fetal period by intrauterine growth charts may not be SGA (Figs. 12.1 and 12.2).

In addition, Doppler velocimetry can detect altered flow states in the fetal-placental and uterine-placental circulation. Among SGA fetuses, it may help to distinguish subjects with IUGR from constitutional SGA. In the first case Doppler flow patterns may be abnormal [2].

Consequently a comprehensive auxological evaluation of the neonate should consider not only anthropometric features at birth but also fetal ultrasound biometry and Doppler velocimetry.

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12.2 Auxological Evaluation of the Newborn

12.2.1 Anthropometric Variables

Weight, length and head circumference at birth are indicators of the quality and quantity of growth. These variables must be evaluated using standardised instruments and following the techniques for accurate measurements described by Cameron [3]. Some anthropometric indexes have been proposed to identify intrauterine malnutrition. They compare variables such as body length and head circumference, which are less influenced by malnutrition, to variables which are more affected, such as body weight. The most well known is the Rörher ponderal index

$$\text{PI} = \text{body weight (g)} \times 100 / \text{length (cm)}^3$$

The normal PI for a full-term newborn lies between 2.2 and 3 (3rd and 97th percentile). This neonatal body proportionality index corresponds to the body mass index (BMI) used in children and adults

$$\text{BMI} = \text{body weight (kg)} / \text{length (m)}^2$$

and is an indirect measure of soft tissue and, by inference, of fat accumulation.

12.2.2 Gestational Age

The validity of neonatal auxological measurement relies on good estimates of gestational age. There is unanimous agreement that the best estimate is obtained by a combination of the reported last menstrual period and early ultrasound assessment [4].

12.2.3 Neonatal Classification

At birth, recognition of growth abnormalities or intrauterine malnutrition is of considerable importance for prognosis and

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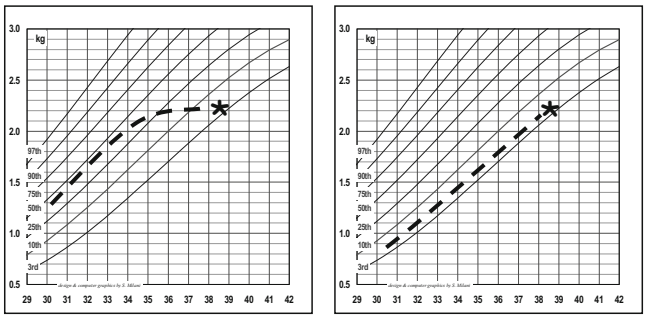
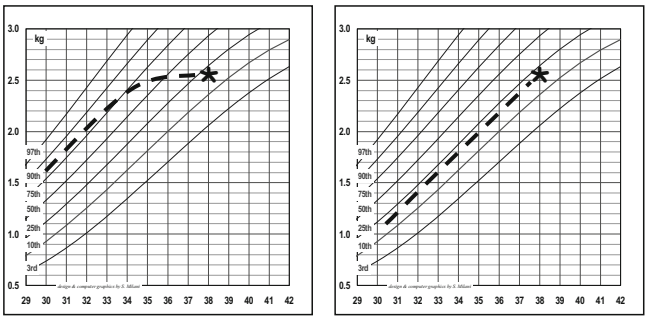


SGA newborn with IUGR SGA newborn without IUGR

Pattern of fetal growth ✱ Neonatal weight

Fig. 12.1 Different fetal growth patterns of two SGA newborns of similar birthweight and gestational age respectively with and without IUGR



AGA newborn with IUGR AGA newborn without IUGR

Pattern of fetal growth ✱ Neonatal weight

Fig. 12.2 Different fetal growth patterns of two AGA newborns of similar birthweight and gestational age respectively with and without IUGR

clinical practice. Neonatal anthropometric charts or curves are used to represent, at different gestational ages, either the percentile values or the mean and standard deviation of anthropometric variables computed from a reference population. A recent commentary defines the characteristics that a reliable anthropometric chart should possess to be of both epidemiological and clinical use (Table 12.1) [1].

Neonatal anthropometric charts enable the classification of newborns at different gestational ages based on weight as follows:

- SGA (small for gestational age): weight below the 10th centile for gestational age
- AGA (appropriate for gestational age): weight between 10th and 90th centile for gestational age
- LGA (large for gestational age): weight above 90th centile for gestational age.

Some Authors set down a cut-off of the 3rd and 97th centile among AGA, SGA, LGA newborns or equivalent limits of mean ± 2 SD [5].

Table 12.1 Characteristics suggested for a reliable neonatal anthropometric chart [1]

– Pre-planned multicentre ad hoc study
– Descriptive reference
– Mono-ethnic population
– Charts specific for gender
– Charts specific for single or multiple pregnancy
– Charts specific for parity
– Reliable gestational age assessment
– Reliable measuring techniques and instruments
– Range of GA from 42 to 24 weeks or less

The American Academy of Pediatrics and the American College of Obstetricians and Gynecologists [4] recognize three groups of gestational age with the following definitions:

- Preterm: born before 37 completed weeks (less than 259 days)
- Full-term: born between 37 and 41 weeks + 6 days (from 259 to 293 days)
- Post-term: born 42 weeks or more after conception (294 or more days).

As a consequence, nine different classes of newborns can be defined.

The definition of small, appropriate and large newborn for gestational age is based on weight. However, similar classes may be defined by body length and head circumference, so that newborns can be defined as having small, appropriate and large body length or head circumference.

Consideration of weight, body length and head circumference traditionally allows for the identification, among the heterogeneous group of SGA newborns, of infants who are symmetrical (proportionate) or asymmetrical (disproportionate). Symmetrical SGA newborns have reduced weight, body length and head circumference.

The insult leading to reduced growth generally occurs early in pregnancy during the phase of growth primarily characterized by cellular hyperplasia with a consequent reduction in cell number (hypoplasia) that may limit subsequent catch-up of organ and tissue growth [2, 6]. Asymmetrical SGA newborns have reduced weight with body length and head circumference that is either normal or reduced to a lesser degree.

The insult causing reduced growth occurs later, interfering with the delivery of oxygen and nutrition during the phase of cellular hypertrophy, resulting in reduced cell size (hypotrophy) and the possibility of subsequent catch-up growth [2, 6]. The weight deficit is principally due to a reduction in fat deposition, particularly during the third trimester of pregnancy. The disproportion is due to the redistribution of blood flow during fetal development with preferential perfusion of the brain, heart and adrenal glands to the detriment of other organs, such as the liver, spleen, kidneys, thymus, and adipose tissue, and growth of the brain and body length are more compromised [7].

In the case of late insults the biggest reduction in fetal weight compared to body length may also be explained by the different dynamics of intrauterine growth: the velocity peak of length growth, about the 20th week, occurring earlier than the peak of weight growth [8]. At six months post conception the fetus reaches 70% of its full-term length, but only 25% of its weight. These newborns, once described as "dysmature", often appear to have clinical signs of intrauterine malnutrition, an appearance that has been likened to that of an "old man", wrinkled, with hypoelastic skin, with skinfolds that can be easily lifted, little subcutaneous tissue and muscle mass, with a thin umbilical cord. When meconium is passed in utero, there is yellow-green staining of fingernails, skin and umbilical cord (Fig. 12.3).

As a result of improved prenatal diagnosis and management of fetal growth restriction, newborn babies with all these features are uncommon these days. The main differences between symmetrical and asymmetrical SGA newborns are presented in Table 12.2.

Some studies have suggested that proportionate and disproportionate SGA newborns represent a continuum rather than distinct classes [9]. More recent data also suggest that both disproportionate and proportionate fetal growth restriction may start early in pregnancy (17–19 weeks of gestation) [10]. These observations have resulted in reconsideration of the causes, timing and risks of symmetric or asymmetric intrauterine growth restriction.

The recognition of specific causes of reduced fetal growth is more important than body proportionality at birth for predicting the short- and long-term outcomes of SGA children.

Growth of the fetus relative to the placenta also seems to be important. Babies born small but with a relatively large placenta are less likely to show catch-up growth during the 18 months after birth and have an increased risk of hypertension in adult life [11].

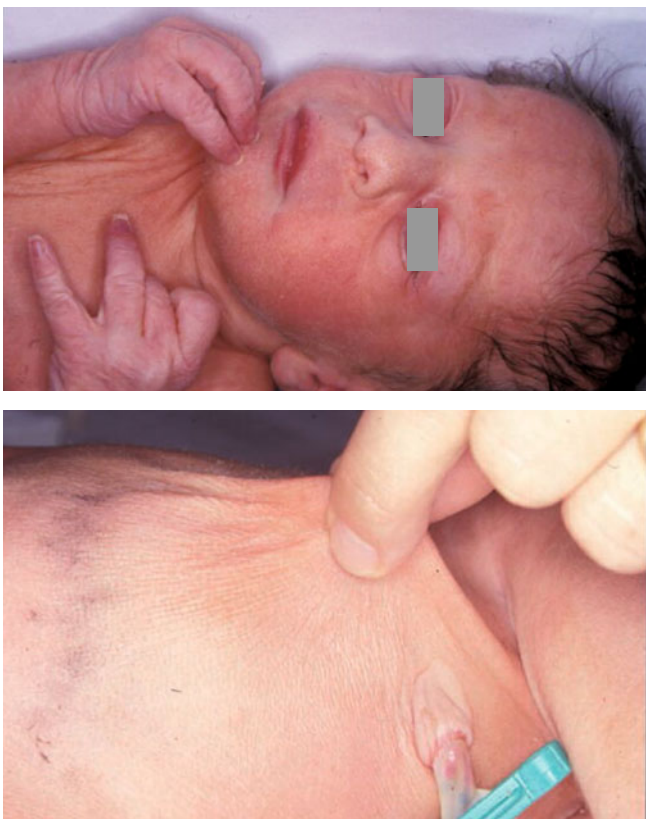


Fig. 12.3 Wrinkled and hypoelastic skin, easily liftable and pliable skinfolds in a IUGR newborn

12.3 Neonatal Outcome

12.3.1 Mortality

Small for gestational age newborns, both preterm and term, have an increased perinatal mortality risk [12, 13]. The increased risk of neonatal and infant mortality observed in late preterm (34–36 weeks) and early term (37–38 weeks) SGA infants persists even when infants with severe congenital anomalies are excluded. In addition to congenital conditions, birth hypoxic ischemia and infections have a relevant role in neonatal mortality whereas sudden infant death syndrome contribute to infant mortality [14].

A higher mortality rate is reported in SGA preterm infants with abnormal (absent or reversed end-diastolic flow) umbilical artery Doppler velocimetry [15].

12.3.2 Asphyxia (Hypoxic Ischemia)

Transient diminished placental blood flow during labor is poorly tolerated by growth-restricted fetuses. Intrauterine chronic hypoxia and limited carbohydrate reserves caused by placental insufficiency are more likely to predispose to perinatal hypoxic ischemia in SGA neonates than AGA newborns with an increased risk of all clinical sequelae of perinatal asphyxia [16].

12.3.3 Infections

Recent studies suggest possible interactions between immunological function and nutritional status. Thymic atrophy and prolonged impairment of cell immunity have been found in SGA infants and animal models of intrauterine growth restriction. These subjects also have a more pronounced hypogammaglobulinemia compared with AGA infants [17]. Neutropenia occurs frequently in infants born to mothers with preeclampsia. All these aspects result in an increased risk of neonatal infections.

Table 12.2 Symmetrical and asymmetrical SGA newborns

	Symmetric (20–30%)	Asymmetric (70–80%)
Onset of fetal growth restriction	Early in gestation	Late in gestation (late second trimester or third trimester)
Pathophysiology	Impaired cell hyperplasia Reduced cell number	Impaired cell hypertrophy Reduced cell size
Anthropometry	Small birth weight, length and head circumference, normal ponderal index	Small birth weight, but relative sparing of length and head circumference growth, low ponderal index
Major clinical problems	Malformations, congenital infections, increased risk of postnatal neurological and growth impairment	Asphyxia, meconium aspiration syndrome, hypoglycemia, polycythemia. Increased risk of type 2 diabetes
Causes	– Genetic disorders and syndromes – Congenital infections – Teratogens exposure – Substance abuse, fetal alcohol syndrome – Constitutional healthy SGA without IUGR	– Impaired uteroplacental function (i.e., preeclampsia, chronic hypertension, long standing maternal diabetes) – Maternal disease (i.e., renal or heart disease, collagen vascular disease, anemia) – Maternal malnutrition – Environmental factors (i.e., high altitude) – Multiple pregnancy – Substance abuse

12.3.4 Coagulation Disorders

The hepatic dysfunction that results from chronic intrauterine hypoxia may lead to hemostatic alterations, mainly concerning a reduction in vitamin-K dependent factors and thrombocytopenia. Such disorders are usually short-lived and respond to simple corrective measures. Occasionally severe bleeding, such as pulmonary hemorrhage, has been reported [18]. In a recent study, prolonged prothrombin time (PT) and international normalized ratio (INR) were observed in full-term healthy SGA neonates, indicating a predisposition to hemorrhagic events. However the newborns included in the study did not have clinical signs of altered hemostasis [19].

12.3.5 Thermoregulation

SGA are at increased risk of hypothermia because of their higher brain weight and body surface area in relation to weight as well as their lower subcutaneous adipose tissue stores, including diminished brown fat reserves that limit nonshivering thermogenesis [20].

12.3.6 Hypoglycemia

Reduced hepatic and skeletal muscle glycogen stores (the predominant source of glucose for the first hour after birth) and impaired glycogenolysis and gluconeogenesis are the main causes of hypoglycemia in IUGR infants. In addition, growth-restricted infants have limited fat stores and do not oxidize free fatty acids and triglycerides effectively, thus failing to spare tissue use of glucose. The risk of hypoglycemia is greatest during the first days of life as the newborn adapts to extrauterine life without a placental source of nutrients, although it may persist for several weeks [18, 21].

12.3.7 Polycythemia

Chronic fetal hypoxia results in increased erythropoietin production and release by the fetal kidney, causing excessive blood red cell production. In addition, a shift of blood from the placental compartment to the fetus during labor or fetal hypoxic ischemia constitute important causes of polycythemia in FGR fetuses [22]. Polycythemia adds to the risk of IUGR infants developing hypoglycaemia, hyperbilirubinaemia and necrotizing enterocolitis (NEC).

12.3.8 Respiratory Distress Syndrome (RDS) and Bronchopulmonary Dysplasia (BPD)

A commonly held view that intrauterine stress associated with IUGR enhances lung maturation has been challenged by reports that the incidence of RDS is inversely correlated with birth weight and gestational age [23], and that growth-restricted infants may have the same or greater [23, 24] incidence of RDS than AGA newborns of the same gestation.

An increased risk of chronic lung disease has also been reported in IUGR preterm infants [25–27]. Multiple etiologies have been implicated for the development of bronchopulmonary dysplasia in SGA. These include abnormal intrauterine lung development [25], oxygen free-radical mediated injury in utero, severe early lung disease with consequent

ventilator-induced lung injury, fetal and/or neonatal systemic inflammatory response due to chronic hypoxia [28].

12.3.9 Retinopathy of Prematurity (ROP)

SGA infants are at increased risk of developing ROP [29]. Changes in organ development due to fetal hypoxemia, nutrient restriction and an altered endocrine environment are all possible explanations [30]. SGA infants are often sicker than their AGA peers, requiring more intensive and prolonged hospital care [20]. They are therefore more likely to require supplementary oxygen, which is a well-documented risk factor for ROP. SGA infants have lower serum concentrations of insulin-like growth factor 1, and there is evidence of roles for this growth factor and for vascular endothelial growth factor in the pathogenesis of ROP [31].

12.3.10 Necrotizing Enterocolitis (NEC)

Infants with IUGR are at increased risk of developing NEC, especially if Doppler studies of flow in the fetal aorta or umbilical artery have identified absent or reversed end-diastolic flow velocities [32]. The underlying mechanism is unclear, but may be associated with alterations in blood flow in the viscera, particularly in the intestine and in the liver. Abnormalities of splanchnic blood flow may persist postnatally with some recovery during the first week, providing justification for delayed and slow introduction of enteral feeds [32]. Early postnatal evaluation of superior mesenteric arterial blood flow has been considered as a tool to identify infants at increased risk of developing NEC [33].

12.4 Long-Term Outcome

12.4.1 Postnatal Growth Impairment

Most term children born SGA experience catch-up growth and achieve a normal adult height, above –2 SD. Approximately 10% of them remain shorter than comparable AGA infants [5, 34]. Catch-up growth is typically an early postnatal process. Term SGA infants usually experience a period of accelerated linear growth during the first 12 months [35] and in most cases catch-up growth is complete by two years [5, 35].

It is has been suggested that SGA children aged 2–4 years with no evidence of catch-up growth and heights less than –2.5 SD should be referred for endocrine evaluation and eligibility for growth hormone (GH) treatment [5].

Preterm SGA infants can take 4 or more years to achieve heights in a normal range. Many preterm infants show a postnatal growth deficit at the time of hospital discharge, so-called extrauterine growth restriction (EUGR), which is defined as a centile at discharge lower than the birth centile. EUGR is largely due to an inadequate postnatal nutrient intake as well as postnatal morbidities, and it increases with decreased gestational age [36, 37].

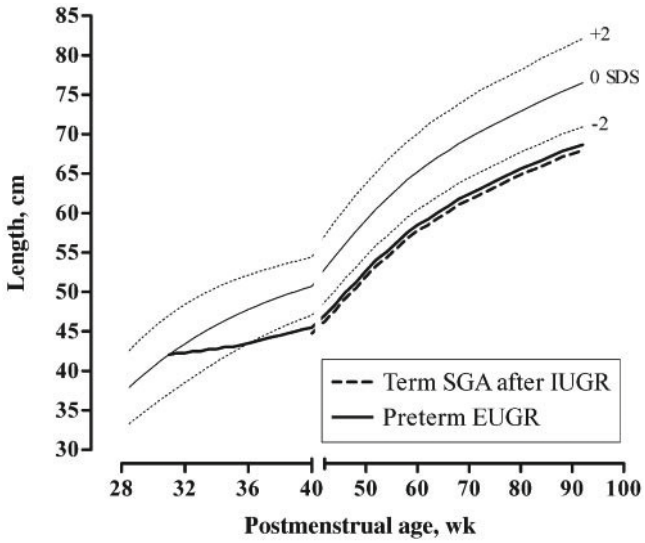


Fig. 12.4 Growth of a term SGA infant after IUGR and of a preterm AGA infant with EUGR. Reproduced from [36], with permission

Premature infants with EUGR also have metabolic abnormalities similar to those observed in term SGA children and these occur irrespectively of whether they are SGA or AGA at birth [38]. Approximately 10% of very preterm children have heights below –2 SD at 4 to 5 years of age. This percentage is similar to that of term SGA infants who do not show postnatal catch-up growth (Fig. 12.4) [36].

SGA term infants suffer from an adverse fetal environment during the last trimester of pregnancy, whereas very preterm infants suffer from an adverse postnatal environment during the first three months, a time biologically equivalent to the third trimester of fetal life.

Considering these similarities, an expert group has recently suggested that the indication of GH therapy should be expanded to preterm children with EUGR whose growth fails to normalize over time [36].

12.4.2 Neurological and Intellectual Risk

There is an increasing evidence that being born SGA is per se associated with mild to moderate learning difficulties in childhood and adolescence, lower psychological and intellectual performance in young adulthood, and low social competence and behavior problems when compared with AGA infants of the same gestation [39]. The most important predictor of subnormal performance is the absence of catch-up growth in height and/or head circumference [5, 16]. Long-term exclusive

breastfeeding could help to prevent some of the neurological sequelae of being born SGA. Overfeeding with an enriched formula could accelerate growth, but it does not seem to lead to an advantage for intellectual development and could increase metabolic and cardiovascular risks (see below) [40, 41]. It has been suggested that GH treatment may improve IQ in short SGA children, but additional data are required [5, 39].

The association between cerebral palsy and IUGR, which has been observed in both term and preterm infants, may be due to placental insufficiency. Reduced oxygen or nutrient delivery to the fetus may have adverse effects on brain development and differentiation [18, 39].

12.4.3 Metabolic Risk

Since the late 1980s, epidemiological studies have shown that impaired intrauterine growth is associated with later development of the metabolic syndrome (MetS) or one of its components (insulin resistance, hyperinsulinemia, impaired glucose tolerance or diabetes mellitus type 2, dyslipidemia, arterial hypertension, and obesity). Additionally, other morbidities have been connected with the syndrome, such as hypercoagulability, non-alcoholic fatty liver disease, renal dysfunction (micro- or macro- albuminuria), polycystic ovary syndrome, endothelial dysfunction, and atherosclerosis leading to increased cardiovascular morbidity and mortality [42].

A number of mechanisms for the development of MetS have been proposed. One hypothesis is that the fetus adapts to an adverse intrauterine environment "reprogramming" the endocrine-metabolic status with benefits for short-term survival. This process consists mainly of insulin resistance and reduced insulin secretion due to the impaired development of beta cells. As a result, blood glucose concentrations can be maintained to benefit brain development at the expense of less glucose being transported to peripheral tissue (e.g., muscle and fat) [43]. This reprogramming maintains glucose homeostasis in the short term when the intrauterine nutrient supply is deficient, but predisposes the child to metabolic syndrome in later life when nutrient supplies are plentiful [18, 35].

SGA newborns show accelerated postnatal growth with rapid weight gain, associated with later obesity, which is per se associated with the MetS [44]. A recent systematic review has shown that both low birth weight and postnatal catch-up growth are correlated with the later development of MetS [45]. Overweight former SGA children also are at increased risk of MetS compared with overweight former AGA children [46].

Poor postnatal growth is associated with later poor neurocognitive outcome. This detrimental effect should be weighed against the metabolic risk that results from promoting faster weight gain [47]. The next challenge for neonatal nutritionists will be achieving "healthy catch-up growth" in IUGR subjects, based on a nutritional strategy for improved neurodevelopmental outcome while minimizing long-term metabolic and cardiovascular adverse effects [45].

12.4.4 Chronic Kidney Disease

When intrauterine resources are restricted, renal development is impaired, possibly at the expense of appropriate development of the brain and heart [7]. The result of this trade-off during organogenesis is a diminished number of nephrons ("nephron underdosing"), predisposing these subjects to albuminuria and to the risk of chronic kidney disease and hypertension. Metabolic syndrome and type 2 diabetes may also cause renal complications [48].

12.4.5 Puberty and Reproductive Function

Girls born SGA have an increased risk of earlier pubertal development or normal timing with rapid progression [49]. These aberrations may result in reduced adult stature. Today there are insufficient data to define the risk of ovarian dysfunction, reduced fertility or early menopause in females born SGA. By contrast, most boys born SGA have normal pubertal timing, but are often short [49]. Although there is sparse information about the influence of fetal growth on gonadal function, most studies have shown that low birth weight is a risk factor for testicular cancer, hypospadias and cryptorchidism [50]. There is little evidence about the influence of fetal growth on adult male reproductive function.

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13

Intrauterine Growth Restriction: Intervention Strategies

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13.1 Introduction

Intrauterine growth restriction (IUGR) or fetal growth restriction (FGR) is defined as a pregnancy in which the fetus fails to achieve his/her genetically determined growth potential or optimal growth. Optimal fetal growth is defined as birth weight achieved in the absence of fetal, maternal or placental factors that can exert a pathological effect on growth [1]. Clinically, whether the fetus or newborn has achieved appropriate growth for gestation is inferred from gender-specific standardized growth charts. IUGR is diagnosed if the weight is below the 10th percentile for a given gestational age. During pregnancy, the diagnosis is made if the estimated fetal weight (EFW) has either fallen below the 10th percentile or if it is on a downward trajectory on consecutive measurements indicating that IUGR can be diagnosed even if the EFW is within the normal percentiles.

The outcome of the IUGR pregnancy is a small for gestational age (SGA) newborn [2]. Thus, IUGR and SGA are not synonymous. IUGR indicates a pregnancy associated with pathology, whereas SGA refers to a newborn whose birth weight is below expected for the gestational age. For both IUGR and SGA, an accurate assessment of the gestational age of the pregnancy is critical for the diagnosis. Although SGA newborns of 34 weeks or greater have mortality rates that are similar to appropriate for gestational age (AGA) newborns, the morbidity (perinatal asphyxia and neonatal transitional problems) remain. The outcomes of SGA preterm newborns (before 28 weeks) are worse than AGA preterm [3]. In addition, SGA infants are predisposed to developing diseases in adulthood including hypertension, coronary heart disease, stroke and type-2 diabetes [4]. This phenomenon, now called "Developmental Origins of Health and Disease (DOHaD)" or Developmental Programming, was first shown in the United Kingdom and has now been replicated among men and women in Europe, United States and India [5].

The interventions to improve birth weights in IUGR pregnancies have not been successful, and to date, delivery at an appropriate gestation is still the most important intervention. In this chapter, the possible diagnostic strategies based on new proteomic technologies and evidence for intervention strategies will be discussed.

13.2 Physiology of Fetal Growth

Normal fetal growth is a series of coordinated cellular processes in various organs and tissues that are regulated by an organism's genome, which is modulated by the cellular microenvironment (gene-environment interaction), which in turn, is determined by fetal, maternal and external environments. The placenta is the critical gatekeeper of the fetal environment and, under normal conditions, it functions to promote development. However, under conditions of limited availability, it may compete with the fetus for its own metabolism [6, 7]. As a target of both maternal (hypertension, renal, coagulopathies) and pregnancy diseases (preeclampsia) it may be damaged, leading to poor function (placental insufficiency).

The cellular processes of growth and differentiation are regulated by complex cell-cell and cell-matrix interactions, which are mediated by locally expressed soluble or structural peptide factors (growth factors, cytokines and matrix). The regulation of the expression of genes that control these intricate processes in various tissues of the embryo/fetus can be categorized into intrinsic (genomic and epigenomic) and extrinsic (environment). The genome of the developing embryo consists of all of the information that is needed to develop from a single cell zygote to a complex multi-cellular and multi-organ/tissue structure of the fetus/newborn and placenta. The epigenetic or epigenomic influence of gene expression is

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

defined as the heritable regulation independent of genomic sequence and includes DNA methylation, histone modification and non-coding RNAs. It is also the major mechanism by which the environment (nutrition, chemicals) regulates gene expression that may be long lasting and sometimes deleterious to the individual's health [8] and may be responsible for developmental programming [9].

13.3 Pathophysiology of IUGR and Intervention Strategies

IUGR remains prevalent (3–10%) in both developed and developing countries, albeit with different etiologies which can be categorized into embryonic/fetal, placental and maternal causes. Clinically, the causes can be categorized into potentially treatable or untreatable. The latter includes genetic (aneuploidy or single gene) and infective (viral or protozoal) causes. The former includes those that are either preventable or managed to reduce mortality and long-term adverse outcomes. In developed countries, placental insufficiency is the major cause, whereas in developing countries, inadequate maternal nutrition and infections (e.g., malaria) play a greater role. The multi-factorial causes of IUGR lead to three possible scenarios: (1) abnormal placental function, (2) inadequate maternal supply of oxygen and/or nutrients or removal of waste, and/or, (3) decreased ability of the fetus to use the supply. The placenta plays an integral role in the first two categories. Abnormal development, inadequate perfusion and dysfunction of the placental villi are often responsible for the development of IUGR, especially in the early onset type, such as seen in severe preeclampsia. Irrespective of the etiology, the final common biochemistry and several molecular pathways include: (1) hypoglycemia and/or hypoxemia; (2) altered insulin and IGF system expression [10]; (3) alterations in the expression or activity of placental transporters, particularly those that transfer amino acids; possibly, the placenta senses its own nutrient supply, alters its transport abilities accordingly, and regulates fetal growth [7].

The interventions depend on the diagnosis, the possible etiology (exclusion of untreatable causes), the assessment of prognosis (continuation of pregnancy to achieve growth and maturity *vs* delivery to prevent intrauterine death) and the effects of intervention.

13.4 Diagnosis and Monitoring

Due to many underlying etiologies of IUGR, a combination of detailed history and examination, fetal, placental and amniotic fluid assessment is required to arrive at the correct diagnosis and management planning. To identify those fetuses that are at risk for adverse outcomes, it is critical to exclude small but normal fetuses, and those in which intervention is not recommended (genetic or infective causes).

13.4.1 Clinical

The standard clinical anthropometric measurements of pre-pregnancy maternal weight and height, and weight gain during pregnancy are still of fundamental importance in the diagnosis of IUGR/FGR. The symphysis-fundal height (SFH) measurement (the distance from pubic-symphysis to the uterine fundus) is a simple, inexpensive and widely used screening tool to detect abnormal fetal growth [11]. The most important aim of SFH measurement is the detection of IUGR fetuses to prevent intrauterine death and the reported detection rate of IUGR ranges from 56 to 86%.

13.4.2 Ultrasound and Doppler

A complementary use of ultrasound (for fetal biometry) and Doppler (of fetal vasculature) methods provides the best assessment of IUGR for both diagnosis and clinical decision making. Detection of IUGR by ultrasound using fetal abdominal circumference or EFW less than the 10th percentile for gestational age and sex is still the most feasible and reliable method.

A detailed and thorough anatomic sonographic survey is essential. A careful study to detect potential aneuploidy markers (e.g., echogenic bowel, nuchal thickening, clinodactyly) and associated congenital anomalies (e.g., omphalocoele, congenital heart defect) can strongly suggest a genetic etiology, and echogenicity of liver or brain can suggest a viral etiology. Serial ultrasound measurements of the fetus and estimated fetal weight, as well as amniotic fluid volume (maximum vertical pocket or four-quadrant amniotic fluid volume estimation) [12] aid in the diagnosis and the timing of delivery.

Doppler assessment of the various fetal vasculature (umbilical vein, ductus venosus and carotid) provides important clinical information on the circulatory changes that are occurring within the fetus and also indirectly provides some evidence of fetal hypoxia or acidemia. The presence of an early diagnostic notch in the uterine arteries at 12–14 weeks is the earliest evidence of abnormal trophoblast invasion, and if present beyond 24 weeks, the diagnosis is almost certain. Reduced umbilical venous flow volume or increased placental blood flow resistance are the earliest signs of reduced villous perfusion. Absent or even reversal of umbilical artery end diastolic velocity indicates that 60–70% of the placental villous vasculature has been damaged with the risk of fetal hypoxemia and potentially acidemia. The late Doppler changes are indicative of advancing circulatory decompensation and potential

impending fetal demise. The pulsatility index of umbilical artery offers a valuable monitoring measurement even in the absence or reversal of the end-diastolic velocity [13].

13.4.3 Biophysical Profile

Biophysical profile is a composite score (fetal tone, fetal breathing movements, gross body movements, amniotic fluid volume and fetal heart rate analysis) that represents the fetal behavioral response to placental insufficiency. The biophysical profile score (BPS) shows a reliable and reproducible relationship with fetal pH [14], however the deterioration in BPS is a later manifestation compared to Doppler changes.

The diagnosis of deterioration of fetal health, using multiple modalities over time, gives the most reliable information. Of all the fetal biometric measurements, the abdominal circumference gives the best measurement with the highest sensitivity and negative predictive value for IUGR, which is further enhanced by serial measurements [15]. Estimated fetal weight (EFW) can also be used as an important monitoring tool, although less sensitive than abdominal circumference (AC) in diagnosing IUGR, it offers greater positive predictive value.

13.4.4 New Biomarkers

Using proteomic technologies, two potential proteins that may be useful as diagnostic and/or prognostic biomarkers – maternal haptoglobin α2 isoforms [16] and fetal site-specific hyperphosphorylated insulin-like growth factor binding protein-1 (IGFBP-1) isoforms have been described [17]. The latter protein is particularly promising as phosphorylation enhances IGF-1 binding and limits IGF-1 bioavailability, and therefore is potentially a biomarker with functional relevance. These markers were identified in IUGR pregnancies after the clinical diagnosis was established, and their usefulness as an early diagnostic or prognostic marker remains to be delineated.

13.5 Management During Pregnancy

13.5.1 Timing of Delivery

The critical decision of delivery of an IUGR fetus is usually made when tests of fetal health suggest that intrauterine risks exceed neonatal risks for adverse outcome. Once the diagnosis is made, the pregnancy is closely monitored for maternal and fetal health, and serial investigations are essential to confirm the diagnosis as well as to monitor for timing of delivery. The principles in management are to avoid fetal demise, ensure optimal fetal maturity, and conduct safest mode of delivery for the best outcome. Recent studies suggest that antenatal glucocorticoids should be used with caution as fetal endogenous glucocorticoids are already elevated in IUGR pregnancies and additional exogenous glucocorticoids may impede maturation [18].

13.5.2 Nutritional Supplementation

Once the diagnosis of IUGR is made, it is usually too late to intervene with any form of nutritional supplementation to enhance fetal growth in the majority of cases. Since the etiology is likely due to the inability of the fetus to grow or the placenta is diseased and is unable to transfer enough nutrients across, nutritional supplementation is usually ineffective. Even in developing countries where maternal malnutrition is the primary cause, nutritional supplementation during pregnancy has not shown to consistently reverse the outcome. Studies to supplement nutrition prior to or early in pregnancy have shown variable results. Nutritional advice to increase energy and protein intake improve gestational weight gain and outcome of pregnancy, but has no effect on the incidence of SGA [19]. Supplementation of micronutrients to pregnant mothers has shown some improvement in birth weight compared to caloric supplementation alone. A systematic review of five trials (1135 women) showed nutritional advice given to pregnant women to increase energy and protein intake improve birth weight, but not the pregnancy outcome [20]. In 13 trials (4665 women), balanced energy/protein supplementation was associated with modest increase in mean birth weight and a substantial reduction in SGA risk, stillbirth and neonatal death. Whether the impact is due to the improvement of maternal health (e.g., correction of anemia) or direct supplementation of fetal nutrition is still unclear. Protein supplementation has not shown to be of benefit, and on the contrary, high protein diet has shown significantly increased SGA risk and increase neonatal morbidity in three trials of 966 women [20].

Hypoglycemia and low insulin levels are common findings in IUGR. However, glucose infusion to achieve euglycemia and increase insulin levels in a fetal sheep IUGR model causes progressive hypoxia and acidosis with increased lactate without an increase in insulin or beta cell mass [21]. Thus, correction of fetal hypoglycemia in IUGR by glucose infusion will not be effective and may potentially be harmful.

Marine oil, which has been suggested to prolong pregnancy and increase birth weight, prolongs pregnancy slightly, by 2.6 days (WMD 2.55 days, 95% CI 1.03–4.07 days; 3 trials, 1621 women) and birth weight was slightly higher (WMD 47 g, 95% CI 1–93 g; 3 trials, 2440 women), but there was no overall change in the incidence of SGA [22]. Supplementation during pregnancy of folate, iron, magnesium, zinc and vitamin D do not improve birth weight or the incidence of SGA [13].

13.5.3 Oxygen Therapy

Fetal hypoxia is commonly present in IUGR pregnancies in which placental insufficiency is the cause and impaired fetal growth is thought to result from reduced oxidative metabolism. Continuous oxygen therapy to mothers from diagnosis until delivery may improve fetal growth and outcome. A systematic review of three studies involving 94 pregnancies indicates lower perinatal mortality rate (risk ratio 0.50, 95% CI 0.31–0.81) and higher birth weights [23]. However, the higher gestational age in the oxygenation group may have accounted for the difference. Further controlled studies are recommended due to the limitation in the number of pregnancies studied so far.

13.5.4 Growth Factors and Growth Hormone

Experimental IUGR in animals is associated with low levels of IGF-I [10]. To reverse the growth impairment, IGF-I has been administered IV or into the amniotic cavity of IUGR in fetal sheep. Both modes of administration significantly increased fetal growth rates, but only the intra-amniotic administration increased liver weight. Growth hormone administration of mothers partially reversed IUGR, however, there was increased the incidence of hydraencephaly [24]. No clinical studies have been performed to date to assess clinical applicability of either treatment.

13.5.5 Low Dose Aspirin and Other Pharmacologic Agents

Some cases of IUGR share placental pathology similar to preeclampsia, and low dose aspirin, which inactivates platelet cyclooxygenase, results in decreased thromboxane synthesis and improved uteroplacental blood flow. A meta-analysis of 13 randomized controlled trials (RCT) including 13,234 women showed a significant reduction in IUGR (OR 0.82, 95% CI 0.72–0.93) and a non-significant reduction in perinatal mortality (OR 0.84, 95% CI 0.66–1.08) [25]. Aspirin was effective at lower dose of 50–80 mg/d but the preventative effect was greater at a higher dose of 100–150 mg/d. However, a more recent meta-analysis of 38 trials showed no reduction in IUGR or perinatal mortality [26].

Other pharmacologic agents that have been proposed to be potentially useful by improving uteroplacental circulation include betamimetics and calcium channel blockers [13]. However, no consistent beneficial effects on birth weight or incidence of SGA have been demonstrated. Less well studied is L-arginine, a nitric oxide donor [27].

13.5.6 Bed Rest

Bed rest is commonly recommended to women with IUGR pregnancies to improve uteroplacental circulation and enhance fetal growth. A systematic review of RCTs revealed only one well conducted study comparing bed rest *vs* ambulation involving 107 women [28]. It showed no difference in birth weight or gestational age.

13.5.7 Exercise

A recent systematic review of regular aerobic exercise (at least 2–3 times/wk) for women during pregnancy included 472 women in 11 trials, although most trials were small and not of high methodologic quality. Exercise improved physical fitness and was associated with a statistically insignificant increased risk of preterm birth (relative risk 1.82, 95% CI 0.35–9.57) with absence of effect on mean gestational age, while effect on fetal growth was inconsistent [29]. Another systematic review of two small good quality trials of 45 women showed no effect on the incidence of preeclampsia (relative risk 0.31, 95% CI 0.01–7.09) [30]. SGA risk was noted in one trial as relative risk 3.0, 95% CI 0.14–64.26.

13.6 Conclusions

Intervention strategies to improve the immediate or long-term outcome of IUGR pregnancies have proven ineffective. The objective of management of such pregnancies is to determine the appropriate gestation and mode of delivery to achieve fetal maturity and avoid perinatal complications. SGA newborns should be closely followed up to determine long-term adverse outcomes. Intervention strategies in the newborn and childhood period are still under investigation.

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14

Late Preterm Infants at Risk for Short-Term and Long-Term Morbidity and Mortality

Avroy A. Fanaroff

14.1 Introduction

Late preterm births were the first group of premature infants who neonatologists treated successfully. Over time with the very low birth weight infants surviving and demanding many resources, near-term infants were relatively ignored and unfortunately were no longer considered to be of high risk by many health care providers. The resurgence of interest in this group, and their renaming to late preterm resulted from the recognition that they were indeed the largest subpopulation of preterm infants, and had an increased mortality when compared to term infants and increased morbidity including transient tachypnea of newborn (TTN), respiratory distress syndrome (RDS), persistent pulmonary hypertension (PPHN), respiratory failure, apnea, temperature instability, jaundice, hypoglycemia, feeding difficulties and a prolonged neonatal intensive care unit (NICU) stay [1–5]. Furthermore, they have an increased prevalence of cognitive and neuro-developmental problems and a greater rate of readmission to hospital in the first weeks after discharge than term births. Escobar noted that many late preterm infants are never admitted to the NICU [6]. A possible explanation is that clinicians may be making clinical judgments based on infants' birth weight only rather than gestation and birth weight.

Preterm births are both emotionally and financially costly, taking a toll on the family and resulting in high direct health care costs due to longer hospital stays and hospital readmissions. Reducing the rate and toll from premature deliveries is both a national and international priority. Because late preterm deliveries, those between 34 and 37 weeks' gestation, account for about 70% of preterm births, they represent the low lying fruit, and are the logical initial target for prevention of prematurity.

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14.2 The Late Preterm Infant

Until recently late preterm infants (those delivered between 34 weeks 0 days and 36 weeks plus 6 days) were regarded and treated as near-term infants. Consequently the considerable morbidity and mortality in this group of infants was largely unnoticed and disregarded. Recognizing that they indeed are the largest group of preterm infants with significant short and long-term morbidity and even mortality, the terminology has changed to emphasize the preterm birth, hence the terminology late preterm, and closer attention is being paid to this specific class of infant. As they account for over 70% of preterm births, utilize excessive resources and as many late preterm infants have no identifiable reasons for their early appearance, they present the best opportunity to substantially reduce the rate of preterm birth [1, 2].

14.2.1 Incidence and Prevention

In the United States, premature births have increased from 10.6% in 1990 to a high of 12.8% of all live births in 2006, declining to 12.7% in 2007 and 12.3% in 2008 [7]. Thirty five states reported a significant decline during the 2 year period. The rise of preterm births during the last three decades was primarily due to a rise in late preterm births from 7.3 to 9.1% of all live births in 2006. In 2006 there were 387,791 late preterm births in the United States, representing 9.1% of live births. Indeed between 1996 and 2006 the rate of infants born late preterm in the United States increased more than 18%. However, there was a slight decline of late preterm infants to 9% in 2007, perhaps reflecting progress in the prevention of late preterm delivery. Late preterm infants, more than 34 and less than 37 weeks' gestation, account for almost three quarters of preterm births (Fig. 14.1.) Almost half of these deliveries are by elective cesarean section. For sound medical reasons and in the best interest of both the mother and fetus/newborn,

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

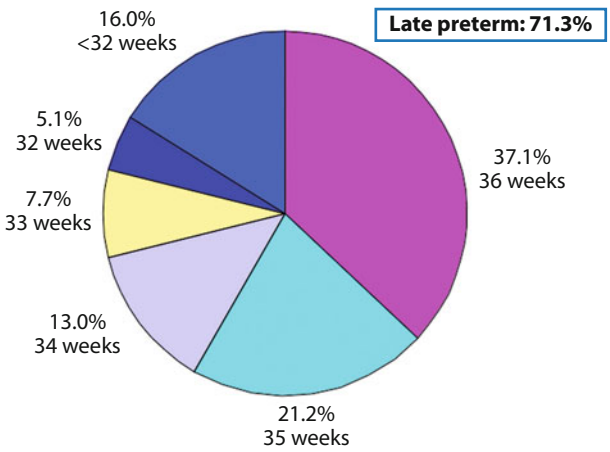

Fig. 14.1 Distribution of all preterm births, USA 2003

The American College of Obstetricians and Gynecologists has recommended that elective deliveries not be performed before 39 weeks of gestation [8].

Davidoff examined the changing epidemiology of gestational length among singleton births in the United States, from 1992 to 2002 [9]. Analyzing gestational age by mode of delivery, the distribution of spontaneous births shifted to the left, with 39 weeks becoming the most common length of gestation in 2002, compared with 40 weeks in 1992 ($P < 0.001$). Deliveries at greater or equal to 40 weeks gestation markedly decreased, accompanied by an increase in those at 34–39 weeks. Singleton births with premature/prolonged rupture of the membranes or medical interventions had similar trends. Changes in the distribution of all singleton births differed by race/ethnicity, with non-Hispanic white infants having the largest increase in late preterm births.

To quantify the adverse neonatal and maternal outcomes associated with elective term delivery at less than 39 completed weeks of gestation, Clark et al reviewed a cohort of 17,794 deliveries, including 14,955 (84%) at 37 weeks or greater. Remarkably, of these term deliveries, 6562 (44%) were planned, rather than spontaneous [10]. Among the planned deliveries, 4,645 (71%) were purely elective, and 17.8% of infants delivered electively without medical indication at 37–38 weeks and 8% of those delivered electively at 38–39 weeks required admission to a newborn special care unit compared with 4.6% of infants delivered at 39 weeks or beyond. Cesarean delivery rate in women undergoing induction of labor was not influenced by gestational age but was highly influenced by initial cervical dilatation and parity, ranging from 0% for parous women induced at 5 cm or greater to 50% for nulliparous women at 0 cm. They rightfully concluded that elective delivery before 39 weeks' gestation is associated with significant neonatal morbidity and is inappropriate.

Oshiro et al [11] reported that before initiation of a concerted system and multidisciplinary team effort (including administrators, information technology as well as bedside care providers such as physicians and nurses) with the goal of reducing elective births before 39 weeks, 28% of elective deliveries were occurring before 39 completed weeks of gestation. Within 6 months of introducing the program, the rate was down to 10% and 6 years later through continued education and a cultural change within the system, the rate is down to a very acceptable 3%.

Until 2007 the rate of preterm births has been increasing in the United States, especially for births at 34–weeks of gestation (late preterm). The causes for these trends remain unclear but significant contributors to these large numbers of late preterm births are elective inductions and cesarean deliveries prior to 37 weeks' gestation. This despite evidence and guidelines recommending against elective deliveries before 39 weeks without clinical indication [8].

Reddy et al [12] characterized the delivery indications for late preterm births and their potential impact on neonatal and infant mortality rates. Using the 2001 US Birth Cohort Linked birth/death files which contained over 3 million singleton births, they categorized delivery indications as follows: (1) maternal medical conditions; (2) obstetric complications; (3) major congenital anomalies; (4) isolated spontaneous labor: vaginal delivery without induction and without associated medical/obstetric factors; and (5) no recorded indication. They noted that among 292,627 late preterm births, the first four categories (those with indications and isolated spontaneous labor) accounted for 77%. The remaining 23% (67,909) were classified as deliveries with no recorded indication. Factors significantly increasing the chance of no recorded indication were older maternal age; race, multiparity; or previous macrosomic infant of more than 4000 g birth weight. The neonatal and infant mortality rates were significantly higher among deliveries with no recorded indication compared with deliveries secondary to isolated spontaneous labor but not surprisingly lower compared with deliveries with an obstetric indication or congenital anomaly. These 23% of late preterm births with no recorded indication for delivery noted on birth certificates present a prime opportunity for preventing late preterm births.

The potential for success in this regard is documented by the Ohio Perinatal Quality Collaborative Writing Committee [13]. They sought to reduce scheduled births between 36(0/7)–38(6/7) weeks that lack appropriate medical indication. They collected baseline data for 60 days from 20 Ohio maternity units and then mandated recording of an indication for scheduled births between 36(0/7)–38(6/7) weeks gestation. Deidentified birth data were analyzed centrally. Rates of scheduled births without a documented indication, birth certificate data, and implementation issues were shared regularly among sites. The rate of scheduled births between 36(0/7)–38(6/7) weeks without a documented medical indication declined significantly from 25% to less than 5% in participating hospitals. Birth certificate data showed inductions without an indication declined from a mean of 13 to

8%. Dating criteria were documented in 99% of charts. Thus merely by establishing and applying dating criteria for gestational age and requiring documentation of the reasons for scheduled birth, the number of late preterm deliveries were substantially reduced. If we can accomplish this beyond 36 weeks there is no reason to believe that it cannot be extrapolated to 34 weeks and beyond with a significant impact on reducing late preterm births. However, in order to sustain the success it will be necessary for scheduled births to be a part of the culture of safety and for mothers to be better educated regarding the risks of late preterm delivery.

Bailit et al [14], utilizing electronic health records, sought to determine maternal and neonatal outcomes by labor onset type and gestational age. They reviewed 115,528 deliveries from 2002 through 2008 categorized by labor onset type (spontaneous, elective induction, indicated induction, unlabored cesarean). Neonatal and maternal outcomes were calculated by labor onset type and gestational age.

Neonatal intensive care unit admissions, the need for assisted ventilation and sepsis improved with each week of gestational age until 39 weeks. The babies did better with elective induction, but there was increased risk of mothers requiring a hysterectomy in that group [14].

14.3 Mortality

In 2002, the neonatal mortality rate (deaths among infants 0–27 days chronologic age) for late preterm infants was 4.6 times higher than the rate for term infants (4.1 *vs* 0.9 per 1000 live births, respectively).This difference has actually widened when compared to 1995 data where there was a four-fold difference between late preterm and term infants (4.8 *vs* 1.2 per 1000 live births, respectively). This relationship also carries over into infant mortality. In 2002, the infant mortality rate, deaths during the first year of life, for late preterm infants was also greater than the infant mortality rate for term infants (7.7 *vs* 2.5 per 1000 live births, respectively) [9, 15].

This is most notable for late preterm small for gestational age (SGA) infants who are 44 times more likely than term appropriate for gestational age (AGA) newborns to die in their first month and 22 times more likely to die in their first year. Even excluding deaths from congenital conditions, including birth defects, the differences in mortality rate ratios persisted for SGA infants, especially those born in the late preterm period.

Thomasek [16] observed that late-preterm infants were particularly more likely to die in the early neonatal period compared with term infants from causes such as respiratory compromise, maternal complications of pregnancy, and congenital anomalies. They noted that infant mortality rates in 2002 were three times higher in late preterm infants than term infants (7.9 *vs* 2.4 deaths per 1000 live births) (Table 14.1). Because many of the deaths were caused by life threatening congenital malformations, which may have precipitated the preterm delivery, the plea for prospective documentation of the indications for late preterm deliveries is timely and appropriate.

Table 14.1 Complications of prematurity in late preterm *vs* term infants

Complication	Frequency late preterm	Frequency term
Jaundice	54%	38%
Sepsis evaluation	37%	13%
Feeding difficulties	32%	7%
Receive IV fluids	27%	5%
Hypoglycemia	16%	5%
Temp. instability	10%	<0.1%
Apnea	6%	<0.1%
Mechanical ventilation	3.4%	0.9%
Mortality (2002)	7.9/1000 Live births	2.4/1000 Live births

Kitsommart [17] compared data on 1193 late preterm, the majority of whom were 36 weeks (44%) followed by 35 weeks (29%) and 34 weeks (27%), respectively, and 8666 term infants. The prevalence of intensive care admission, respiratory support, pneumothorax, and mortality in late preterm infants was significantly higher compared with term infants. Although only 1% had positive cultures, almost 30% were treated with antibiotics. Despite a low overall mortality rate of 0.8%, the late preterm group had a 12-fold higher risk of death. The increased mortality and morbidity thus confirm the high-risk status of late-preterm infants.

14.4 Morbidity

It is estimated that nearly 50% of infants born at 34 weeks' gestation require intensive care, this number drops to 15% at 35 weeks and 8% at 36 weeks gestation. In order to identify infants at the highest risk for life threatening morbidity and those most likely to require specialized care Shapiro-Mendoza et al [15] classified a newborn as having a morbid condition during initial hospitalization when at least one of the following three criteria was met: (1) newborn hospital stay of 5 nights and any morbidity diagnostic code considered life-threatening, (2) newborn hospital stay of 5 nights and transfer to a higher level medical facility, and (3) infant death before hospital discharge. They reported that the risk for newborn morbidity increases two-fold with each earlier week of gestation, beginning at 38 weeks' gestation, until 34 weeks' gestation (Fig. 14.2). Late preterm infants are at greater risk for newborn morbidity than term infants, especially when maternal morbidity also is present. This risk seems to be particularly intensified when an infant has had prenatal maternal exposure to hypertensive disorders of pregnancy and antepartum hemorrhage.

Late-preterm infants were seven times more likely to have newborn morbidity than term infants (22 *vs* 3%) (Fig. 14.2).

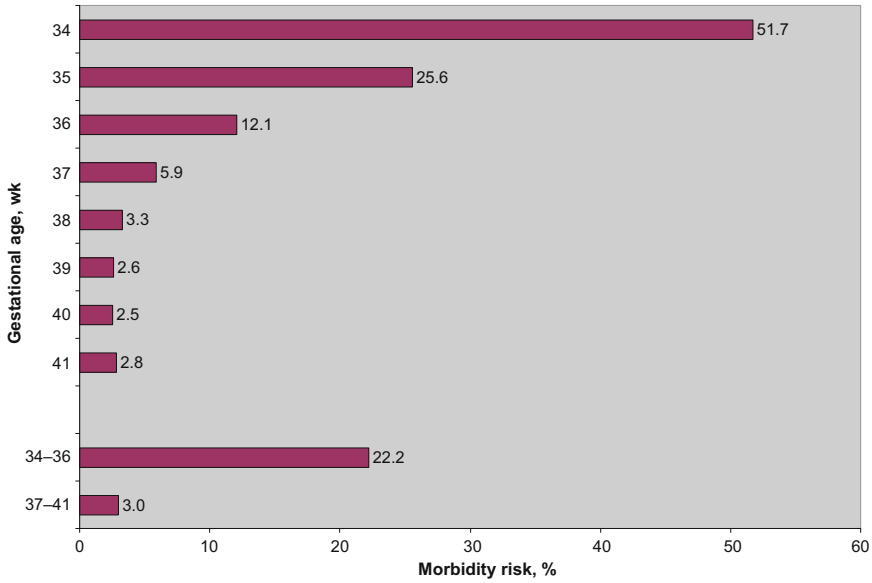

Fig. 14.2 Proportion with newborn morbidity during birth hospitalization according to gestational age. From [15], with permission

Late-preterm infants who were exposed to antepartum hemorrhage and hypertensive disorders of pregnancy (HDP) were especially vulnerable. Additionally HDP, diabetes, and asthma, are associated with an increased risk for indicated or spontaneous preterm birth (Table 14.2).

Decisions regarding the delivery of late preterm infants take into account the risks and benefits to both mother and fetus of prolonging the pregnancy. Specific standards of care and protocols are necessary for late preterm infants rather than applying those developed for term infants. Furthermore better anticipation, recognition and treatment of women with chronic and pregnancy-related health conditions may decrease the rates of newborn morbidity in all infants but especially in late preterm ones.

The major causes of morbidity encountered in late preterm infants include transient tachypnea of newborn (TTN), respiratory distress syndrome (RDS), persistent pulmonary hypertension (PPHN), respiratory failure, apnea, temperature instability, jaundice, hypoglycemia, feeding difficulties and a prolonged neonatal intensive care unit (NICU) stay [20]. In addition they have a higher prevalence of congenital malformations which, as noted above, may contribute to an increased mortality relative to term infants. They are also more likely to develop blood stream infections (Table 14.1).

Table 14.2 Risk for infant morbidity term *vs* late preterm [15]

Maternal condition	RR ratio LPT/Term
Hypertensive disease	6.1
Diabetes	5.4
Antepartum hemorrhage	5.1
Acute or chronic lung disease	6.1
Maternal infection	4.4
Cardiac disease	5.7
Renal disease	4.6
Genital herpes	10.0

14.5 Readmission

Escobar evaluated the large Kaiser Permanente data base which included 33,276 survivors of neonatal intensive care with 862 (2.6%) < 34 weeks' gestation, 2153 (6.5%) between 34 and 36 weeks, and 30,261 (90.9%) of more than 37 weeks [6]. Rehospitalization rates within 2 weeks after nursery discharge varied by gestational age range: 26/862 (3.0%) among babies <34 weeks, 94/2153 (4.4%) among babies 34–36 weeks, and 618/30 261 (2.0%) among babies 37+ weeks. Hence there is a two-fold risk of rehospitalization for late preterm infants compared with term infants and jaundice was the major reason for readmission. These data are substantiated by McLaurin, who reported readmission rates over the first year of life for late preterm infants were 15%, compared to 8% in term infants [19]. In particular, late preterm infants are three times more likely to be admitted within the first 15 days after discharge from the birth hospitalization (3.8 *vs* 1.3% [19]. The most common reasons for early readmission are jaundice, feeding difficulties, poor weight gain, dehydration, and apnea. Respiratory (including bronchiolitis) and gastrointestinal disorders are the most common diagnoses for late readmission (≥ 15 days after the date of discharge) during the first year of life. Underwood et al noted that the largest cohort of infants readmitted to the hospital at least once during the first month of life were infants born at 35 week's gestation, most commonly due to a respiratory illness [20]. The cost of hospital readmission for this cohort of infants approached 100 million dollars.

14.6 Thermal Instability

Whereas the late preterm infant is better equipped than the extremely low birth weight and premature infant, they are still vulnerable to cold exposure after delivery. Increasing amounts of fat are deposited in the final weeks of gestation so they have less white fat and brown fat accumulation and maturation, notably the controlling hormones surge at term. Late preterm infants often have temperature instability, which in turn leads to the consideration of sepsis, a sepsis evaluation and often the initiation of antibiotic therapy [21].

14.7 Transient Tachypnea of the Newborn

A major respiratory disorder in late preterm infants is transient tachypnea of the newborn (TTNB). TTNB is best described as the consequence of delayed and inadequate lung liquid clearance. This self-limiting disease, characterized by an increase in respiratory rate, occurs more frequently in infants delivered by elective cesarean section and is thought to result from delayed activation of liquid clearance in infants not subjected to the stress of labor. Gowen and others showed that newborn infants with TTNB had a transient decrease in amiloride sensitive nasal epithelial Na^+ transport compared with normal newborns, supporting the notion that suboptimal clearance of liquid is a cause of TTNB [22]. Infants with TTN were found to have low norepinephrine but normal epinephrine levels.

14.8 Respiratory Distress Syndrome

With more liberal use of antenatal corticosteroids commencing in 1994, and the introduction of surfactant in the early 1990s, infant deaths associated with respiratory distress syndrome decreased by 48% from 1989–1991 to 1995–1997 and then decreased by another 18% by 2002–2004 in the United States. The latter mortality reduction was evident at 28–32 weeks but not 33–36 weeks of gestation because these infants have not been optimally targeted to receive prenatal corticosteroid prophylaxis, despite evidence from randomized trials regarding the safety and efficacy of the treatment at this gestation. As corticosteroid use is infrequent beyond 33 weeks' gestation (15%) "addressing the knowledge practice gap in corticosteroid use at 33 and 34 weeks should reduce infant morbidity and mortality" [23].

Extrapolating the data from infants born at 24–32 weeks gestation, Joseph et al [26] concluded that the number needed to treat estimates for 35 weeks (n = 21) and 36 weeks (n = 64) of gestation are sufficient to warrant additional attention in terms of future research addressing efficacy at these more advanced gestational ages. However meta-analyses reveal non significant protective effects beyond 36 weeks [23].

Dudell et al [24] reviewed the ELSO Neonatal registry to study the indications for and outcomes of late preterm infants requiring extracorporeal membrane oxygenation (ECMO). The cohort comprised 2258 late preterm infants, representing 14.5% of 15,590 registered neonates. The primary etiology of hypoxic respiratory failure in late preterm infants treated with ECMO was RDS or sepsis as compared to term infants who were more likely to have aspiration syndromes. The overall survival (74%) has not improved over time, and was inferior for the late preterm infants as compared with 87% in the term population.

14.9 Apnea

Apnea occurs more frequently in late preterm infants (4–7%) than in term infants (1–2%) [25]. In the Collaborative Home Infant Monitoring study (CHIME), the recorded rate of apnea (obstructive and central apnea) and bradycardia events was greater in late preterm infants *vs* term infants [25, 26]. Late preterm infants may also be at increased risk for sudden infant death syndrome compared to term infants.

14.10 Feeding Difficulties

Late Preterm infants may have immature feeding mechanics with poor coordination of sucking and swallowing resulting in limited fluid intake and dehydration, often necessitating intravenous therapy. It is also more difficult to establish breast feeding and there may also be accentuated gastroesophageal reflux in late preterm infants. The American Academy of Pediatrics (AAP) has recommended that prior to discharge it is essential to establish successful feeding defined as coordinated sucking, swallowing, and breathing while feeding [2]. Weight loss should not exceed 7% of birth weight during birth hospitalization. If the infant is breast fed, twice daily documented observation by trained caregivers of successful position, latch, and milk transfer after birth also should be performed.

14.11 Jaundice

Hyperbilirubinemia is the most common clinical condition requiring hospital readmission during the first week of postnatal life. Late preterm infants are at substantially higher risk for severe hyperbilirubinemia than infants born at term. Infants born at 36 weeks' gestation have almost an eight-fold increased risk of developing a total serum bilirubin greater than 20 mg per 100 mL (5.2%) *vs* those born at 41 or 42 weeks gestation (0.7 and 0.6%, respectively) [27]. Approximately 1 in 650–1000 infants > 35 weeks' gestation develop total serum

bilirubin values greater than or equal to 25 mg per 100 mL and 1 in 10,000 have levels greater than 30 mg per 100 mL.

The risk factors for readmission are documented in Table 14.3 and are dominated by gestational age and breast feeding [28]. The jaundice may be attributed also to early discharge, inappropriate follow-up and monitoring as well as diminished fluid intake related to poor feeding. Late preterm infants accounted for 40% of kernicterus in a large series reported by Bhutani and Johnson [29]. All were discharged early and breast fed.

Most vaginal deliveries, including the late preterm infants, are discharged by 48 hours of life, well before the peak serum bilirubin that is observed in term infants on day 3–5 and later in preterm infants. The peak bilirubin thus occurs at home. It is therefore necessary to screen the bilirubin levels prior to discharge and arrange for early follow-up either at home or in the clinics to prevent catastrophic non monitored elevation of serum bilirubin. Decisions are made on the bilirubin levels according to age in hours and zones according to the Bhutani nomogram [29, 30].

It has long been accepted that jaundice progresses in a cephalo-caudal fashion as the total serum bilirubin (TSB) level increases. Kramer provided the first systematic study of this process by dividing the body into five zones ranging from zone 1 (jaundice restricted to the head and neck) to zone 5 (jaundice in the hands and feet) [1]. As the jaundice progressed from the head to the extremities, the TSB levels increased, although there was a wide range of bilirubin values in each zone [31]. To evaluate the visual assessment of jaundice Keren et al [32] utilized experienced well baby nursery nurses to visually evaluate newborns for jaundice at a mean age of 47 hours. The objective was to determine whether the pre discharge visual assessment would help to predict the subsequent risk of significant neonatal hyperbilirubinemia, defined as a bilirubin level that exceeded or came within 1 mg/dL of the AAP phototherapy treatment threshold [33]. Those whose pre discharge jaundice was seen in zones 4 and 5 were five times more likely to develop significant hyperbilirubinemia than those with no jaundice, or jaundice restricted to zone 1. Nevertheless, the correlation between cephalo-caudal progression and the ability to predict the risk of hyperbilirubinemia was highly erratic. An exception was the complete absence of jaundice (zone 0), which was present in only 17% of the population but had a powerful negative predictive value of 99%. The conclusion is that it is dangerous to rely on visual assessment of jaundice and if there is clinical evidence of jaundice then the bilirubin should be measured either chemically or by transcutaneous measurement. The AAP guidelines are directed at reducing the frequency of severe neonatal hyperbilirubinemia (levels >17 mg per 100 mL) and bilirubin encephalopathy, in addition to minimizing parental anxiety, discontinuation of breastfeeding and hoping to reduce costs. The key elements of the guidelines are presented in Table 14.4.

Table 14.3 Risk of being readmitted for phototherapy*

Risk factor	Odds ratio (*vs* 40 weeks)
35–36 wks	13.2
37–38 wks	7.5
Breast feeding	4.2
Jaundice in nursery	7.8
Length of stay < 72 h	3.2

* 30,000 discharges from well baby nursery 1988-1994; 4.2/1000 readmitted for phototherapy [28].

Table 14.4 AAP Jaundice Guideline The 10 key elements [33]

1. Promote and support successful breastfeeding
2. Establish nursery protocols – include circumstances in which nurses can order a bilirubin
3. Measure TSB or TcB if jaundiced in the first 24 hours
4. Visual estimation of jaundice can lead to errors, particularly in darkly pigmented infants
5. Interpret bilirubin levels according to the infant's age in hours
6. Infants < 38 weeks particularly if breast fed are at high risk
7. Perform risk assessment prior to discharge
8. Give parents written and oral information
9. Provide appropriate follow-up based on time of discharge and risk assessment
10. Treat newborns when indicated with phototherapy or exchange transfusion

14.12 Long-Term Outcomes

Until recently, the assumption was that late preterm infants carry minimal risk for long-term morbidities. Morse et al [34] refuted these assumptions when they compared prekindergarten and kindergarten outcomes among healthy late preterm infants, and healthy term infants. Outcomes were adjusted for 15 potential confounding maternal and infant variables. They observed that the risk for developmental delay or disability was 36% higher among late preterm infants compared with term infants. Furthermore the risk for retention and disability in kindergarten with need for special education was higher for late preterm infants. The assessment "not ready to start school" was borderline significant. Thus healthy late preterm infants compared with healthy term infants face a greater risk of developmental delay and school-related problems up through age 5 [35].

On the other hand Gurka et al [36] applied eleven standard outcomes measuring cognition, achievement, social skills, and behavioral/emotional problems using the Woodcock-Johnson Psycho-Educational Battery-Revised and the Child Behavior Checklist, administered repeatedly through age 15 years. They detected no consistent significant differences between late preterm and full-term children for these standard measures from ages 4–15 years. They concluded that late-preterm infants born otherwise healthy seem to have no real burdens regarding cognition, achievement, behavior, and socio-emotional development throughout childhood.

14.13 Discharge Planning

Clinicians who care for late preterm infants need to first recognize and establish that the infant is a late preterm infant. They must be aware of their increased risk for morbidity, mortality and potential neuro-developmental problems. They must anticipate, screen for and intervene in an appropriate and timely manner for the common problems encountered in these vulnerable infants. Prior to hospital discharge, parents need to be educated that their infant is at increased risk for jaundice, feeding difficulties, hypoglycemia and dehydration. Parents must be taught to recognize these conditions and promptly seek appropriate care following hospital discharge.

The AAP has established the following guidelines for discharge criteria for late preterm infants [2]:

- Determine the accurate gestational age, and ensure that there are no abnormalities or medical condition (i.e., hyperbilirubinemia) that requires further hospitalization.
- The infant demonstrates physiologic stability by demonstrating competency in the following:
  - Thermoregulation defined as an axillary temperature of 36.5–37.4ºC in an open crib.
  - Cardio-respiratory control with stable vital signs of a respiratory rate less than 60 breaths per minute and a heart rate between 100 to 160 beats per minute, and absence of medical illness.
  - Passed at least one stool spontaneously.
- Completed other routine newborn care. This includes screening tests (i.e., hearing and disorders that are threatening to life or long-term health), vaccinations (i.e., hepatitis B vaccine), and prophylactic treatment (i.e., vitamin K prophylaxis).
- Assessment of the family and home environment to identify any risk factors that may impact on the health of the infant.
- Successful training of the parents who have demonstrated competency in the care of their infant and the ability to assess for hyperbilirubinemia, feeding difficulties, and dehydration.
- A follow-up visit 24–48 hours after discharge is scheduled with an identified primary care provider.

14.14 Summary and Recommendations

In the United States, late preterm infants, defined as infants with a gestational age between 34 to 36 weeks and 6 days, account for 9% of all births.

Late preterm infants have a reported seven-fold increased risk of morbidity compared to term infants during birth hospitalization, which results in a longer hospital stay and higher medical costs. The most common causes of morbidity include hypothermia, hypoglycemia, respiratory distress, apnea, hyperbilirubinemia, and feeding difficulties. Late preterm infants have a higher mortality than term infants. Readmission rates are two to three times greater for late preterm compared to term infants. Early readmission (within 15 days of discharge from birth hospitalization) includes hyperbilirubinemia, feeding difficulties, poor weight gain, dehydration, and apnea. During the first year of life, respiratory and gastrointestinal disorders are the most common diagnoses for late readmission. Late preterm infants also have more neuro-developmental disabilities and school problems than their term born peers.

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15

Ethical Problems

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15.1 Introduction

"I will prescribe regimens for the good of my patients according to my ability and my judgment and never do harm to anyone." The Hippocratic Oath expressed beneficence and non-maleficence as basic principles of medical ethics more than 2000 years ago. Beneficence in perinatal and neonatal medicine refers to the responsibility of the physicians to act in the "best interests" of the fetus and newborn infant. Non-maleficence relates to the avoidance of harm to the fetus and infant, but also to the mother. This implies that the benefit of treatments should outweigh the harm and risks of the treatment for the patient.

In the newborn infant, benefits are usefully defined as survival and the ability to live a self-determined life. For infants with no chance of survival, aggressive intensive care is not beneficial, but harmful and futile, and is, therefore, generally assumed to be unethical. However, in infants with uncertain prognosis (i.e., high risk of survival with poor quality of life), the decision is extremely difficult and requires clear ethical guidelines.

A recent meta-analysis on decision-making in the best interests of the newborn demonstrated that prejudices play an important role in the attitudes (and hence decisions) of caregivers [1]. Janvier et al [2] criticized that neonatal intensive care is usually far more scrutinized than intensive care of any older patient groups and that children and adults with a poor prognosis are more likely to be resuscitated than newborn infants with the same or a better prognosis. The authors explain the different attitudes by deficient knowledge about the actual outcomes or by irrational negative associations with low gestational ages. To avoid decision-making by prejudices and confirmation bias, evidence-based medical ethics applies research results (particularly epidemiology) in addition to ethical and legal principles to draw conclusions to ethical dilemmas in medicine.

Three groups of newborn infants are usually considered in end-of-life decisions: 1) infants at or below the gestational age limit of viability; 2) infants born with congenital anomalies incompatible with life; and 3) extremely ill preterm or full-term infants who might survive with severe damage of the brain or other vital organs after a long period of intensive care. This chapter focuses on the extremely preterm (EP) infant and reviews some of the important issues, such as outcome data, withdrawal and/or withholding life support, role of parents and health care providers, economics, and a framework for neonatal ethical issues.

15.2 Survival and Outcome of Extremely Preterm (EP) Infants

15.2.1 Survival of EP Infants

The knowledge of published survival and long-term outcome data is of great importance for antenatal and postnatal counseling of the parents and for the decision process in the care of EP infants. The prognosis depends on several factors such as gestational age, birth weight, gender, single or multiple births, prenatal and postnatal care, neonatal complications and morbidities, attitudes of the caregivers and parents, and on the individual maturity and resilience of the fetus and infant (Table 15.1).

Gestational age is accepted as major prenatal predictor of the prognosis of EP infants [3] since prenatal care (transfer to a perinatal center, corticosteroids) and antenatal counselling of the parents are mainly based on the gestational age. Most laws and guidelines on the limit of viability in EP infants use a defined gestational age as a limit. However, the estimated gestational age may deviate by ±7 days from the actual gestational age due to uncertain last menses and errors

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Table 15.1 Determinants of the outcome of extremely preterm (EP) infants

Fetal factors
- Gestational age
- Body weight
- Gender
- Single or multiple births
- Social status and lifestyle of the mother
- Individual maturity and resilience of the fetus

Antenatal and intra-partum care of the fetus
- Maternal transfer to a level III centre
- Antenatal steroids, tocolysis, antibiotics, etc.
- Cesarean section on fetal indication
- Experience in "proactive care" of the mother and the EP fetus

Neonatal factors
- Individual maturity, strength and resilience of the infant
- Sufficient maturity of vital organs for survival (lungs) and for intact survival (brain, eyes)
- Condition of the EP infant at birth and response to resuscitation within 3–5–10 min
- Stability/instability in the first 12–24 postnatal hours
- Extent and required time of intensive care
- Neonatal complications and severe morbidities (IVH/PVL, ROP NEC, sepsis)

Neonatal and long-term care of EP infants
- Experience in resuscitation and neonatal intensive care of EP infants
- Availability of adequate equipment, resources and experienced staff around the clock
- Neonatal developmental care
- Specialized long-term care
- Socio-cognitive and learning support

Policies, attitudes and individual decisions
- Policies and guidelines concerning the care of EP infants
- Attitudes of the obstetrical and neonatal staff towards the care of EP infants
- Antenatal decisions of parents and caregivers (initiation and withholding of intensive care based on risk factors of death and long-term sequelae)
- Postnatal decisions of parents and caretakers (withdrawal of intensive care if the prognosis of the infant deteriorates)

of prenatal ultrasonography, unless the time of (*in vitro*) fertilization is precisely known [4].

A recent review of 20 published studies on survival rates of EP infants born between 1985 and 2008 [5] shows that live-born preterm infants with a gestational age of 22 weeks had an overall survival rate of 8% (145/1791) with no consistent trend to improvement with time. Survival rates ≥20% were noted in Japan [6–8] and in some single centre studies. In Japan, infants born at 22 weeks' gestation are principally considered viable and resuscitated. From 2004 to 2007, 1-year survival of infants born alive at 22 to 26 weeks of gestation in Sweden was 70% and ranged from 9.8% at 22 weeks to 85% at 26 weeks [9]. The overall survival rate of infants born at 23 weeks' gestation was 25% (1361/5383). Eight studies showed improvements and 6 showed decreases in the survival rates with time. Survival rates above 50% were reported for Sweden [9] and Japan [6–8].

Preterm infants born at 24 weeks' gestation showed an overall survival rate of 50% (3796/7555). A Japanese study on infants born in 2005 showed the highest survival rates in the whole-country studies (77%) [8], followed by Sweden in 2004–2007 (67%) [9], England and Wales in 2005 (56%) [10], and Norway in 1999–2000 (55%) [11]. In eleven time trend studies, a marked rise of the survival rates was observed with time. The overall survival rate of infants with 25 weeks' gestation was 68%. Japan, Sweden, Norway, and England and Wales reached again high survival rates of 85%, 81%, 77%, and 76%, respectively. Overall survival rates increased from 61% in infants born mainly in the 1990s to 74% in infants born mainly in the early 2000s. Infants born at 26 weeks' gestation had an overall survival rate of 80% [5].

15.2.2 Neonatal Morbidities and Long-Term Outcome in EP Infants

Seven recent studies on severe neonatal morbidity in surviving EP infants <27 weeks presenting week-by-week results have been reviewed elsewhere [5]. At 23 weeks' gestation the prevalence of intraventricular hemorrhage and/or periventricular leukomalacia ranged from 9 to 29%, at 24 weeks from 14 to 28% and at 25 weeks from 7 to 15%. Infants with 23 weeks' gestation had a high risk of severe retinopathy (17 to 62%) requiring surgical treatment. Blindness resulting from retinopathy of prematurity (ROP) has, however, become rare due to modern laser coagulation techniques.

The high risk of EP infants for neurodevelopmental impairments, such as cerebral palsy and cognitive or sensory deficits are of great concern to parents, caregivers and society. Only few national and regional studies present week-by-week outcomes in EP infants [5]. Severe disability was noted in 20–22%, 8–39%, and 11–27% of infants born at 23, 24 and 25 weeks', respectively. No disability was seen in 13–33%, 16–56% and 17–52% in infants born at 23, 24 and 25 weeks' gestation, respectively. Two Japanese studies showed relatively low rates of neurodevelopmental disability in infants born at 23 weeks' gestation (32–35%) [12, 13]. EP infants without visible disability in early years are at considerable risk for a variety of behavioral and educational deficits at school age.

There is concern that newly developed treatments and altered attitudes towards EP infants improve their survival rate, but that many of the additional surviving infants suffer from severe disability. A rising prevalence of long-term deficits such as cerebral palsy and poor cognitive outcome was indeed observed in several time-trend studies on EP infants born in the 70s and 80s and in some studies in the mid 1990s and the early 2000s [5, 14]. Recent studies showed both increasing survival rates and decreases in the rates of short- and long-term neurodevelopmental disabilities in EP infants [14]. The recent improvements of short- and long-term outcomes are probably the result of a comprehensive antenatal and postnatal care

philosophy including proactive antenatal care, optimal delivery procedure, competent neonatal care in the delivery room and the intensive care unit, better long-term care and a positive attitude of more caretakers towards these patients [15].

15.2.3 Prediction of the Outcome of Individual EP Infants

The prognosis of EP infants depends on several factors in addition to the gestational age. Although it is difficult to consider all of these factors in counseling the parents and in decision-making, obstetricians and neonatologists should be aware of the complex risk profile in individual infants. Several models have been developed that consider some or several of the factors influencing the short- and long-term prognosis of EP infants. Several guidelines include the option of withholding treatment if the infant's condition appears poor immediately after birth. This agrees with the attitude of many neonatologists who want "to see what the infant looks like" if the infant is born at the limit of viability [16]. However, several studies demonstrated that the infant's condition at birth (low Apgar scores and low heart rates at 1 and 5 min, need of cardiopulmonary resuscitation) did not predict poor outcome in EP infants [17, 18]. Thus, the condition at birth is an unreliable predictor of the prognosis of EP infants, unless resuscitation of more than 3–5 min is required to stabilize an EP infant.

Definitions of the limit of viability and of the "grey zone" in EP infants [19–22]:

a. Viability is defined as the possibility to live and survive outside the uterus independent of the risk for severe neurodevelopmental disability.
b. Viability is determined as a reasonable chance to survive without "severe and unacceptable" morbidity (usually defined as severe neurodevelopmental disability).
c. Viability is defined as the ability to grow and develop normally outside the uterus, the ability to communicate and to engage in meaningful relationships with others, to become a child and later achieve independent moral status and personhood.

The outcome of infants born at 22 weeks' gestation is actually extremely poor; infants born at 23–24 weeks' gestation have an uncertain prognosis, and infants born at 25 weeks' gestation have a reasonable chance to survive without severe disability. The lower limit of viability is currently set at 22–23 weeks, and the "grey zone" at 23 0/7 to 24 6/7 weeks. Infants born in the "grey zone" have an uncertain prognosis with high risk of death or survival with severe long-term deficits, but the individual prognosis depends on a variety of biologic and individual (maturity, strength and resilience), technologic and general care factors (including antenatal proactive care, attitudes of staff and developmental care in the NICU). The "grey zone" also considers the uncertainty of the assessment of gestational age by approximately ±7 days [4].

15.3 Laws and Guidelines for the Care at the Limit of Viability

The World Health Organisation (WHO) defines a life birth as "a fetus, whatever its gestational age, exits the maternal body and subsequently shows any sign of life, such as voluntary movement, heart beat, or pulsation of the umbilical cord, for however brief a time" [23]. The term "live birth" was defined by the WHO mainly for public health, legal and statistical purposes and should not be used synonymously with "viability". Signs of life may already be seen in extremely immature infants born at 20 weeks of gestation and no chance of survival.

The "Born-Alive Infant Protection Act" enacted by the US government in 2002 obliges the physician to "protect infants who are born alive at any stage of development". Enforcement guidelines to the law issued in 2005 requires the hospital and its medical staff "to perform a medical screening examination on that born-alive infant" and to treat the infant if signs of life are detected [24]. The legal conditions in the US may explain that the guidelines of the American Academy of Pediatrics [25, 26] recommend withholding life support in infants with 22 weeks' gestation, but do not give detailed recommendations for more mature infants. Similar guidelines have been issued in Spain [27]. In Italy, the National Bioethics Committee reccomends resuscitation of all babies born alive when a possibility of survival exists [28].

Some national laws define the limit of viability to address primarily the limit of legal abortion (e.g., Japan and Italy: 22 weeks; Netherlands, Singapore and UK: 24 weeks). In Japan the viability limit has been lowered from the previous 24 weeks' gestation to 22 completed weeks' gestation in 1991 (Motherhood Protection Act) based on surveys of survival rates in EP infants [6]. As a result of the legislation in Japan, intensive care is provided to all EP infants born at ≥22 weeks' gestations [13].

In most western countries, as well as in Japan and Australia, guidelines regarding the limit of viability have been established by medical organizations in agreement with national laws and court rulings [6, 22, 29–40]. The guidelines usually present recommendations for the care of infants with extremely poor, uncertain and fairly good prognosis. No country recommends resuscitation of infants with gestational age below 22 weeks. For infants of 22 weeks' gestation, guidelines of five countries recommend "comfort care only" [25, 30, 31, 34, 36]. The other guidelines propose individual decisions based on parental request or the infant's condition at birth. For infants with 23 weeks' gestation, three countries recommend "comfort care only" [31, 36, 39], while the other guidelines recommend individual decision or care according to parental request. At 24 weeks' gestation, four guidelines regard intensive care as due according with individual decisions [33–35, 40], four as generally indicated [6, 22, 37, 38]. At 25 weeks' gestation, most guidelines regard intensive care as "generally indicated". Resuscitation on the basis of gesta-

tional age has also been criticized [41]: The likelihood of a favorable outcome with intensive care can be better estimated by considering four factors in addition to gestational age: sex, exposure to antenatal corticosteroids, single or multiple birth, and birth weight [42].

15.4 Quality of Life and the Best Interests of the Infant

Assessments of the quality of life, the harm/benefit ratio and the best interests of the infant are often made to decide whether it is appropriate to begin or to continue intensive care. In the decision-making process the estimated quality of life corresponds to the probable benefit resulting from medical care and the best interests are deducted from the harm/benefit ratio. Parents and professionals caregivers are particularly concerned with the risk of "brain damage" and what it means for the child. In the newborn infant, quality of life is usually related to the estimated future health and development. There are several concerns with the definition of the future quality of life in preterm and severely sick neonates:

- The future quality of life is usually estimated from risk factors such as gestational age, birth weight, gender, signs of brain damage in ultrasound scans and other complications during intensive care. Quality of life is thus estimated from individual factors that are compared with statistical data studied in a cohort of infants with similar risk factors. The epidemiological approach is objective, but uncertain for the individual child.
- The meaning of an estimated future quality of life in infants cannot be judged by the infants themselves, but is estimated by the families, the caregivers and the society. It includes effects of the health status on lifestyle in the children and their families, the quality of life probably experienced by the child and the value placed by the public, the family and the child on compromised health states.
- The uncertainty of the estimation of the future quality of life is difficult to bear for many parents and caregivers, as the consequence of the judgment may be the death of the infant. This is admitted in some countries, but cannot avoid the suspect of a conflict of interests: some parents can overestimate babies' future disability or they cannot accept even a low-grade handicap that, on the other hand, the baby might bear.
- Life-and-death decisions based on the judgment of the quality of life have been regarded discriminatory against handicapped persons. Many disabled persons might consider their quality of life as good or acceptable. Some can argue that the decision to withhold or withdraw intensive care is only made in infants with anticipated disability that will probably lead to intolerable suffering and burden, and inability to interact with others, but some authors do not agree.

Several national laws, decrees, court rulings and guidelines request that any decision made on behalf of a person lacking decision-making capacity must be in the person's "best interests". Three major interests of the premature or sick neonate have been defined [43–45]:

- Interests in the neonate's health: reasonable care using recognized standards of care and appropriate individualized care measures have to be provided.
- Immediate (short-term) interests of the infant: pain and suffering, harm and indignity should be avoided by the selection of appropriate therapeutic measures (e.g., developmental care, pain treatment, comforting care).
- Long-term or future-potential interests of the infant: these are usually defined as good or acceptable long-term outcome.

In addition to the infant's interests, familial, communally, and public (moral and legal) interests play a role [43, 44]. The family members have their own interests and incorporate religious and cultural values. Communal or social interests consider cultural beliefs about the value of disabilities, concerns for dignity and respect. Public interests include protection of citizens from abuse or neglect. Medical care of newborn infants has to be compatible with moral and legal duties to incapacitated individuals.

Pain and suffering from intensive care procedures (present quality of life) are related to the risk for long-term suffering and burden (future quality of life). If the infant has no chance of survival, intensive care will merely prolong pain and suffering, and natural death may be in the best interests of the baby. If the outcome of the infant is uncertain, but most likely associated with poor health and development, the best interests will be judged from both the present pain and suffering (intensive care) and the estimated future suffering and burden.

Financial considerations should not play a role in the judgment of the best interests of an infant with uncertain prognosis by the caregivers and the society. The best interest concept is mainly applied in uncertain situations, but may be uncertain itself. What is the probability of survival of an individual infant? What is a reasonable, an acceptable and an unacceptable long-term outcome? Kipnis [46] defined three types of uncertainty in the estimation of the outcome: The vagueness of the definition of "intolerable deficits", the uncertainty of whether a baby will benefit from an intensive care intervention or be harmed by it, and the inability to determine the harm-to-benefit ratio of (sometimes aggressive and painful) intensive care measures. Moreover, the application of the best interest standards may be flawed by limited knowledge or lack of consensus about treatment choices, conflicts of interests, personal beliefs, bias and prejudice, lack of empathy, ignorance or disregard of persons' rights and duties [1, 44].

Critics of the "best interests" concept argue that it is vague, open to abuse, and permits decision-makers to do whatever they think is best. Decisions may be made in the interests of the parents and caregivers rather than in the best interests of the infant. To overcome the uncertainties of the best interests

of an invidual infant, it has been suggested that a best interests' standard should incorporate what a reasonable person of good will would want or consider acceptable in similar circumstances ("reasonable person standard" [43, 44]). Physicians and other health professionals should be aware that in clinical practise it is impossible to know the true best interests of an infant or to define the ideal best interests for an infant. The consideration of evidence-based facts, the clinical intuitions at predicting the outcome of an infant, has been found useful in one study [47]. In some countries the decision to withhold or withdraw intensive care in an infant is up to the parents. The best interests of the infant should be judged jointly by the parents and the physicians. The physicians do not make the final decision, but rather provide the parents with all the information they need do make an explicit decision. This information includes the probable outcome, the necessary intensive care procedures to achieve this outcome and the anticipated burden (including the social and financial burden).

Saigal and colleagues reported in a cohort of adults who were born extremely low birth weight, survivors had no difference in self ratings of health-related quality of life (HRQOL) when compared with normal birth weight controls [48, 49].

15.5 Attitudes Towards Extremely Preterm and Critically Sick Neonates

Attitudes of caregivers towards withholding and withdrawing intensive care of EP and critically sick infants with uncertain prognosis vary among different health professions, among different countries and regions, among various centres of a country or region, and among different caregivers in one centre [15, 50, 51]. Moreover, attitudes of caregivers and parents may deviate considerably. Attitudes of obstetricians and pediatricians were similar in most studies, while nurses in some countries tended to be more prone than physicians to withhold resuscitation in the delivery room and to consider parental opinion regarding subsequent treatment choices than physicians [35, 52, 53]. Nurses frequently underline the suffering of the newborn, whereas physicians often stress uncertainty in treatment outcome [54].

The EURONIC study group revealed that most caregivers in seven European countries would limit intensive care in EP infants with fatal/terminal illness, but that 45–60% of the responders from Germany, Italy and Spain would not set limits in case of poor neurological prognosis [51]. Moreover, the attitude towards "explicit involvement" of parents in decision-making varied between 10% (France) and > 90% (UK). If the physicians wished to discontinue intensive care of an infant with poor prognosis, approximately 50% would continue intensive care at parental request.

Bellieni and Buonocore [1] reviewed attitudes of caregivers concerning the best interests of the newborn. They conclude that several aspects of the personal (age, gender, having own children, fear of litigation), social, religious and professional background (position, experience, knowledge, number of cots) influence the attitudes of the caregivers. Religious convictions play an important role in the attitudes towards care of EP or severely critically infants independent of the denomination [50, 55].

Janvier and colleagues demonstrated that attitudes towards the care of EP or critically sick newborn infants deviate markedly from the attitudes towards older children and adults with similar or worse prognosis [2, 56]. Asked if they would resuscitate patients with identical outcome (50% risk of death and 50% risk of severe neurodevelopmental disability in survivors), 69% of the respondents would resuscitate an EP infant, 87% a full-term neonate and 97% a two-month-old infant. The authors conclude that EP infants are considered "morally different from older children" [57] and speculate that the majority of the respondents had either irrational negative associations with low gestational ages or they were unaware of actual outcomes [58].

Table 15.2 summarizes several indications that neonates, in particularly EP infants, are valued differently from older children and adults. A positive attitude of all prenatal and postnatal caregivers towards the care of a high-risk infant is

Table 15.2 Facts and causes of devaluation of the lives of preterm and full-term infants

- The fetus has almost no rights in utero, but suddenly becomes a citizen after birth
- Termination of pregnancy is legal in many countries even after the limit of a preterm's viability
- The extremely preterm infant is frequently described as "living fetus" or "fetal infant", thereby implying the same lack of rights for the newborn infant as for the fetus
- Infants of older mothers or mothers conceived by *in vitro* fertilization are often called "precious" or "irreplaceable" children in contrast to apparently less precious other infants (e.g., of very young mothers). This relativity of the value of a preterm infant is unthinkable for a critically sick child
- Some ethicists believe that newborn infants lack "personhood" (personality) and consciousness and are thus comparable with an animal rather than with a human child or adult
- There is widespread belief that bonding of infant and mother is less in newborn infants than in older infants and children and even less in premature infants
- Many caretakers are unaware of actual outcomes of extremely preterm infants and overestimate mortality and disability rates and the costs of care
- Irrational negative associations with low gestational ages are frequent in caregivers and the public. Confirmation bias results in the selection of arguments (and research results) that confirm the assumption of poor prognosis of extremely preterm infants
- Uncertainty of a prognosis (e.g., extreme prematurity, intracranial hemorrhage, severe asphyxia or malformations) may justify withholding and withdrawal of treatment in a neonate, but not in an older infant or child
- The informed decision process of the parents requires reliable information at a time when the caretakers themselves are still depending on subjective interpretation of benefits, risks and burdens of the infant

an important determinant of the prognosis. If some physicians or nurses think that an EP or severely sick infant is not viable, they might treat the baby that way, resulting in self-fulfilling prophecy. Education of all staff involved in prenatal and postnatal care of high-risk infants about the actual outcomes using valid epidemiological data, and elimination of irrational negative associations with low gestational age or other risk factors are important prerequisites for a good outcome of high-risk infants.

15.6 The Rights of the Newborn and the Parents

Mercurio distinguishes three rights of the newborn, the right to life, the right to mercy and the right to justice or fair treatment [59]. The right to life is part of many national constitutions and includes the right to appropriate medical treatment. He defined the right to mercy as "a right not to be subjected to pain or discomfort that is very unlikely to yield benefit". The right to justice or fair treatment includes the right to equal medical treatment independent of age, ethnicity, income, etc. Different attitudes to the care of preterm, full-term and older infants [58] violate this right, if they influence the care. The right to justice and equal treatment includes the use of proper epidemiological data in the decision to treat or not to treat an EP infant.

Most countries grant parents the legal rights to decide on behalf of their minor children including the general upbringing, the education, the religion and medical treatment decisions. The parents have the right to get all available information on the condition of their sick child and on therapeutic options for their child. After they have received all necessary information, the parents have the right to select from the therapeutic options, but also to refuse treatment or to have the infant transferred to another hospital. It would be inappropriate to override parents' wishes, since they are usually motivated by their infant's best interest, have natural authority over their child, express religious views in their opinion and bear the burden of treatment decisions [60].

On the other hand, the parents do not have the right to refuse necessary and reasonable treatment. Thus, parents' rights are rebuttable when their decisions pose a significant risk to the life or well-being of the infant [60]. "Parents have a right to make martyrs of themselves, but not of their children" [59]. Moreover, parents have no right to demand treatment that is of no benefit to the child. Without perceived benefit to the child, the parental right to decide is outweighed by the child's right to mercy [59]. These limitations of parental authority in decision-making for their preterm or sick infants do not justify to bar parents from accurate information about the long-term prognosis, resuscitation and treatment options. In many hospitals parents still have limited informed input, even if the infant is born in the "gray zone" of viability [51, 61].

15.7 Informing and Counselling Parents

Guidelines in many countries give parents the major responsibility for the decision-making in EP infants in the gray zone. This responsibility requires comprehensve and accurate information on the prognosis of the infant and treatment options before and after birth.

The major purposes of antenatal and postnatal counselling are: 1) to inform the parents accurately about the condition, the reasonable treatment options and the prognosis of the fetus or the newborn infant, and 2) to assist the parents with decision-making [19, 62, 63]. The consultations have to be honest, fair and compassionate, sensitive to the culture and religion, the level of understanding, the physical and psychological condition of the mother and the father. Translation services may be necessary for parents not proficient in the national language. Senior physicians together with other experienced staff should perform the consultations in a spirit of fairness and compassion. If the counseling physicians are sure that the parents have understood the information, options of care (inclusive withholding and withdrawing of intensive care) should be presented to the parents. Both parents should be present at the consultation, unless the father is legally or for other reasons not involved. In this case the mother should be given the possibility to have a confidant involved. If the mother is under-age or incapacitated, the mother's parents may share the authority with their daughter [64].

Antenatal counseling may be necessary for women with a high risk of delivering an EP or critically sick infants within days or weeks and for women with imminent delivery of a high-risk baby. The prenatal consultation is a collaborative process that should involve obstetricians and midwifes, neonatologists and neonatal nurses, and (if necessary) pediatric subspecialists, pediatric surgeons, and genetic counselors [65]. Communication among various specialists is of great importance to avoid inconsistent and contradictory information and counselling [62]. A shared decision between parents and physicians requires a trusting relationship between parents and caregivers. The parents should be encouraged to consult their family doctor or other health professionals outside the hospital, discuss their situation with relatives and friends, and be offered support by clergy, psychologist or social worker.

The first antenatal consultation is usually performed by the obstetrician and/or midwife and focuses on antenatal treatments (selection of hospital, tocolysis, corticosteroids, cesarean section). Parents should be given accurate information about the chance of their infant surviving and surviving without severe disability. Many physicians believe that the amount of specific medical information is too much for the parents. However, parents have the right to receive the same information as the physicians to make far-reaching decisions for their child. Framing of the information may influence the decision of the parents: Persons who were informed on the prognosis of an EP infant born at 23 weeks' gestation as having a chance of intact survival chose resuscitation of an EP

infant more often than parents who were given prognosis as risk of death and disability [66].

A neonatologist should become involved as early as possible to describe the anticipated immediate and long-term problems after birth, short-term and long-term prognosis, the expected extent of resuscitation and subsequent intensive care. Current information on outcome data of EP infants should include the prognostic relevance of the gestational age, birth weight, gender, multiple birth and prenatal care [42, 67]. Moreover, the parents should be informed on local care possibilities and on local outcome data in comparison with referral centres. Statistical outcome data presented in counseling should include risk of death and risk of survival with severe/moderate/mild impairments (including their meanings). It should also be mentioned that "normal" surviving infants are at risk of a variety of behavioral and educational deficits. Financial obligations are of little importance in counseling and resuscitation in most countries [68], but should be considered if the prognosis of the infant is poor and the financial costs for the family are high.

The anticipated quality of life, pain and suffering of the infant from intensive treatments, but also the burden for the family should be addressed. Moreover, the parents should be informed about the option to withdraw intensive care if the treatment becomes futile. During the consultation, the attitudes and preferences of the parents should be evaluated to assess the best interests of the infant as judged by the parents. An experienced neonatal nurse should explain nursing procedures in the NICU (including feeding of mother's milk, kangaroo care, developmental care) and (if possible) give the mother and/or the father a tour of the NICU. If possible, the consulting neonatologist(s) and neonatal nurse(s) should be part of the resuscitation team in the delivery room and the initial intensive care team for the infant. If this is not possible, the counselors have to make sure that the resuscitation team is informed on the decisions made before delivery and does not oppose to the decisions, unless the situation has changed considerably.

In countries where the decision is given to the parents, there is controversy as to whether counseling of parents should be directive or non-directive [63]. In non-directive counseling, information on outcome data is given (including the uncertainty of the data) without recommendation for the decision and parents are asked to decide whether they want their infant resuscitated after birth. This gives the parents full authority, but also the entire responsibility for a life-or-death decision. They might feel left alone with their decision, worries and emotions and may later regret their decision and feel guilty. Kaempf et al [69] demonstrated that shared authority of the staff and parents using clear guidelines for counseling are well accepted by the parents.

For many parents detailed information on the prognosis and discussion of treatment options are less important than religion, spirituality, and hope guided decision-making [70]. These parents may be reluctant to make a decision, but may prefer to leave "things in God's hands" and want everything done. Different moral and religious attitudes may be a source of conflicts between physicians and parents. Dunn [71] proposed to ask five questions, particularly if the views and decisions of the parents do not agree with the medical staff:

- Do the parents understand the clinical condition and prognosis of their child?
- Do they wish or should they consult others before finally making up their minds?
- Do they require more time?
- Is their decision a loving, caring one made in the interests of their child?
- Are they asking to pull life-saving treatment only for their baby's interest?
- Is their decision a reasonable one in the interests of their child and, if in doubt, is it so unreasonable as to request them to seek other medical advice or for me to take legal steps to take the child into care under the protection of the courts?

Breaking bad news is particularly challenging and requires the sensitivity and communication skills of the physician. If bad news is given with warmth and affection and parents are treated with respect and sensitivity, most parents will have positive memories and will be grateful to the staff involved [72].

15.8 Decision-making in EP Infants

Decisions concerning the care of EP infants may be required at three times:

1. Care of the mother and the fetus before birth. Parents and physicians have to decide whether antenatal proactive care is provided (transfer of the mother to a perinatal centre, tocolysis, antenatal corticosteroids, Cesaren section on fetal indication).
2. Initiation or withholding of resuscitation of the infant after birth. The decision as to whether the infant will be resuscitated is made before birth, but may be changed according to the infant's condition and the infant's response to resuscitation. The decision to provide proactive antenatal care is usually combined with the decision to resuscitate the infant and vice versa.
3. Continuation or withdrawal of neonatal intensive care. Reorientation of care may be considered if provisional care of an infant with assumed poor prognosis has been unsuccessful or if the prognosis deteriorated (e.g., large cerebral bleeding with inactive electric brain activity). The decision to withdraw intensive care in some countries should be made jointly by parents and staff.

The reasons for end-of-life decisions have to agree with national or hospital guidelines. Palliative care treatments (e.g., analgesic drugs) have to be compatible with national laws and court decisions. In most guidelines no or small chance of survival ("futility" of treatment) and no or small chance of survival without severe disability are recognized conditions. The

entire team (including subspecialists as pediatric neurologists, etc.) and (as required) the Ethical Committee should become involved in the end-of-life decision.

If the prognosis is uncertain, the harm-to-benefit ratio (i.e., pain and suffering from intensive care, suffering and burden from long-term impairments in relation to probable long-term outcome) should be estimated and considered in decision-making. Most guidelines consider only severe disability leading to intolerable suffering and burden and/or inability to interact with others as justification for withholding and withdrawal of life sustaining treatment. Whether suffering and burden resulting from intensive care and disability are intolerable has to be decided by the parents. However, the decision to withhold or withdraw intensive care is primarily a medical decision for which the doctor bears ultimate moral and legal responsibility.

The major concern of an end-of-life decision is that the outcome of an individual infant is estimated from statistical data determined in a group of (more or less) representative infants studied several years earlier. Because of the uncertainty of the prognosis, some authors are strictly against any end-of-life decision made before birth, but rather opt for an individualistic approach based on the infant's clinical condition and initial response to intervention at birth [28]. However, most authors agree that "extending intensive care to all of the most immature infants would entail considerable suffering, resource use, and cost in order to benefit only a small proportion of infants" [67]. This can be considered as a utilitarian viewpoint, but it is the logical consequence of dealing with an uncertain situation in which judgement and decision have to be made in the assumed best interests of the individual infant.

When parents are involved in end-of-life decision, the following scenarios may occur after birth in infants whose prognosis was uncertain before birth:

1. If a joint decision of parents and physicians has been made before birth to resuscitate the infant, resuscitation of the infant should usually be tried even when the condition of the infant is severely compromised at birth.
2. If a joint end-of-life decision of parents and physicians has been made before birth, resuscitation of the infant will usually not be attempted. If the infant appears viable (spontaneous respiration), resuscitation should be started within 1–2 minutes to avoid severe hypoxia.
3. If no joint decision of parents and physicians has been made before birth because the mother was already in active labour when admitted to the hospital or because of other reasons (e.g., language), the neonatologist must decide on behalf of the infant and should in case of doubt resuscitate the baby until the parents can be involved in the decision.

Parents differ in their request to be supported in their decision by the staff. Some parents want staff to suggest to them what to do, others wish to receive the necessary information but prefer to make decisions independently or with the support of family, friends, family doctor or religious adviser [72]. Although most parents wish support, the staff members have to avoid any devaluation of parental autonomy and self-worth. "Most parents want to feel guided, assisted and listened to, but not directed or controlled, and certainly not abandoned" [72]. Parents will usually make concerned and loving decisions in their infant's interests.

In countries where the decision to withhold or withdraw intensive care is taken with the essential statement of the parents, the main causes of conflicts between parents and physicians are different opinions of the condition and best interests of the infant, insensitive behavior and communication of the team, and religious convictions of the parents that forbid withdrawal of life-sustaining treatment [73, 74]. Parents usually make concerned and loving decisions in their infant's interests. A Dutch analysis of disagreements in end-of-life decisions demonstrated that conflicts within the team occurred in 4% and between the team and the parents in 12% of the cases. All conflicts were resolved by postponing the end-of-life decision until consensus was achieved [74]. When treatment is clearly futile, the physicians can decide to stop intensive care without decision of an Ethics Committee or court in some countries [75]. In case of conflict between parents and neonatologists that is the parents request to withdraw treatment, while the team sees a realistic chance of survival without severe disability and wishes to continue intensive care, intensive care should generally be continued.

15.9 Withholding and Withdrawing of Intensive Care

15.9.1 Withholding vs Withdrawing of Intensive Care

In various studies end-of-life decisions preceded death in 70–80% of dying newborn infants [76–77]. Principally three ways of ending or limiting intensive care are possible with the intention to shorten the life of a patient: 1) Withholding of resuscitation and intensive care after birth; 2) withdrawing of life-support; and 3) withholding of additional therapies (e.g., antibiotics, vasoactive drugs). The third way is preferred by many professionals and parents, but may result in survival with additional damage (e.g., from sepsis, hypoxia). Although probably not uncommon, withholding of additional therapies is rarely mentioned in reports on end-of-life decisions [76].

Several guidelines see no moral or ethical difference between withholding and withdrawing intensive care [19, 26, 32, 33, 40, 77]. Some authors explicitly opt for withdrawal rather than withholding life support, since "provisional" treatment allows for evaluation of the clinical condition of the infant, including the actual gestational age and body weight, and the reaction to resuscitation and intensive care [40, 77]. Boyle [78] describes the widespread American attitude: "If the treatment in question never is started, no one will benefit. If it is started, some may benefit, and it can be withdrawn from those

whom it does not benefit". During "provisional" intensive care a harm-to-benefit ratio can be estimated repeatedly to determine whether it is appropriate to continue or not. Some national guidelines (e.g., Swiss) recommend "provisional care" of EP infants born at a gestational age in the "gray zone" [40].

However, some religious convictions allow withholding, but not withdrawing of life support [73]. Furthermore, for many caregivers and parents it is psychologically more difficult to withdraw than to withhold intensive care measures. The EURONIC study demonstrated that neonatologists in Italy, Spain, France and Germany consider it more acceptable to withhold than to withdraw mechanical ventilation, whereas in the Netherlands, the UK and Sweden more than 93% of the neonatologists would withdraw intensive care in selected circumstances [51]. Reasons for reluctance to withdraw intensive care include fear of legal consequences, increasing emotional attachment of parents and infants with time, and feeling of active life-ending or even killing by extubation and application of pain and stress relieving drugs.

On the other hand, for some parents the death of their child may be more bearable if the neonatal team has "tried everything" and "gave him or her every chance" [59]. Moreover, a period of intensive care allows some time for the parents and siblings to meet the child, to perform baptism or other religious ceremonies, to touch and cuddle the baby. The infant may die in the arms of the parents and the family has time to grieve with their child and to mourn in dignity. Some parent-infant bonding may relief the mourning sorrow. These possible emotional benefits must be weighed against painful intensive care measures and the use of intensive care resources [79].

15.9.2 Withholding Proactive Antenatal Care and Resuscitation after Birth

Proactive antenatal care (transfer to a center, corticosteroids, cesarean section on fetal indication) improves the prognosis of an EP infant to the level of a 1–2 weeks more mature infant [8, 15, 40, 67]. It is therefore reasonable to decide either for both active antenatal care and postnatal resuscitation or for neither of these active approaches, unless the postnatal condition of the infant requires reorientation of the decision made before birth. Some parents might prefer a passive approach before delivery, but active life-support when the infant appears viable after birth. Even for preterm infants with a gestational age below the limit of viability, an experienced neonatologist should be present or immediately available at birth to confirm or to reorient the medical decision made before birth. However, a joint decision of the parents and physicians made before delivery should not be reversed after birth without marked change in the expected clinical situation.

When resuscitation is futile, compassionate care must be provided as for babies whose intensive care is discontinued. The baby may be placed on the breast of the mother and die in peace. If the baby suffers, an analgesic and/or sedative drug should be given. An appropriate religious ceremony should be offered and organized.

Since EP infants requiring more than 3–5 minutes of resuscitation have a poor prognosis [16–18, 80], the ILCOR guidelines [81] suggest to stop resuscitation "if there are no signs of life after 10 min of continuous and adequate resuscitative efforts". In clinical practice 10 minutes may, however, be a rather brief resuscitation time, since ventilation and other resuscitation measures are not always adquate [81]. A framework of care of extremely preterm infants derived from various guidelines and personal experience of the author is shown in Table 15.3.

Table 15.3 Framework of care of extremely preterm infants (< 26 weeks' gestation)

General considerations
– Extremely preterm infants deserve the best state-of-the-art care to attain the best possible outcomes. Withholding treatment because the hospital cannot provide the appropriate care is unacceptable. If experienced obstetricians and neonatologists are not available at the hospital, the mother has to be transferred to a specialized centre. If a transport is not possible, the most experienced local staff should be involved and get advice from a specialized centre
– Accurate estimation of gestational age (as a major determinant of the prognosis) is extremely important. Moreover, body weight, gender and multiple births influence the prognosis and should be considered in antenatal counseling of the parents
– Parents' rights to comprehensive information and explicit involvement in decisions about antenatal care (transfer to level III centre, tocolysis, corticosteroids, cesarean section) and postnatal care of their preterm infant born before or in the "gray zone" (23–24 weeks' gestation) have to be respected
– A decision before birth not to resuscitate or to resuscitate may have to be reconsidered after birth due to inaccurate prenatal estimation of gestational age and individual variation of the maturity and viability of the infant. The heart rate response of the baby to ventilation within 3–5(–10) minutes is a better indicator of the prognosis than the clinical condition at birth

15.9.3 Withdrawing of Intensive Care

The decision to withdraw intensive care is usually made in several steps.

1. The medical decision of treatment futility or intolerable long-term suffering is made collectively by the NICU team and other subspecialists (e.g., pediatric neurologist, cardiologist or surgeon).
2. The parents are informed on the prognosis of their infant and explicitly involved in the judgment of the best interests of their infant. Moreover, the parents are informed about options to prevent pain and suffering when their baby dies, and they are involved in the decision of appropriate pain and stress relieving treatment. Parents are supported in seeking advice by medical professionals outside the

hospital, friends and religious advisers. In some countries parents are requested to take an active role in this decision.
3. If parents and caregivers agree on the decision to withdraw intensive care, a time is set for ending intensive care (usually assisted ventilation). The parents decide whether they wish baptism or other religious rituals in correspondence to their faith.
4. Intensive care is reoriented to compassionate and palliative care. If possible, the baby is cared for in a private family room. Invasive procedures, blood sampling and any other painful or stressing interventions should be avoided or minimized. Maintaining dignity, warmth, skin contact (kangaroo care), relief of pain and stress are basic elements of palliative care [19, 82]. A family-centered care philosophy is an optimal basis for neonatal intensive care and for the care of a dying infant. Family members and friends are given time and opportunity to stay with the infant, to touch and cuddle the baby.
5. Withdrawal of life-sustaining treatment should take place in an unhurried, private and dignified manner. In the presence of the parents, tubes and electrodes are removed and the infant dies in the arms of the parents. Pain and stress relieving drugs should be given and the bronchial and gastric tube should be sucked to avoid vomiting and choking (if possible, before the parents enter the room). A nurse and a physician (with a good relationship to the parents and the child) should be present until the infant has died and should see the family from time to time thereafter.
6. The staff shows empathy and supports the family in their grief. Mementos of the infant such as photos and videorecordings, armbands, the clothes, a footprint and a strand of hair may be given to the parents. Parents should be supported with funeral arrangements and formalities as death certificate. Some countries allow taking the dead baby home for some time before the funeral. Further consultations (outside the NICU!) should be offered. Information about bereavement and parents groups should be provided.

15.9.4 Pain and Stress Relieving Drugs

For intensively treated newborn infants with a good prognosis, indications and dosages of pain reducing drugs are carefully adjusted to avoid serious side effects. After an end-of-life decision has been made, side effects are of little importance and the dosages can be increased to reach complete elimination of pain and stress. Moreover, pain and stress relieving drugs are indicated to minimize pain and suffering resulting from the removal of tubes, lines and electrodes and from dying itself. Several authors recommend administration of an adequate dose of opiate analgesia (morphine or analogues) together with a sedative (e.g., benzodiazepine) as part of humane care. Although the required high dosages of analgesics and sedatives may cause respiratory depression and shorten the life of the infant, their use is considered legal in most countries as long as the primary intention of the physicians is relief of pain and suffering and not shortening the life of the infant [83].

The administration of lethal dosages of drugs with the intention to hasten death is illegal in most countries. In The Netherlands, "non-voluntary euthanasia" is considered acceptable in selected infants for whom death appears to be in the infants' best interests, but who are presently not dependent on life-support. The Groningen Protocol for Euthanasia in Newborns gives detailed guidelines that are far more restricted than most guidelines and recommendations for withholding or withdrawing of intensive care [84]. In fact, only 4% of infants with end-of-life decision (born in The Netherlands from 2005–2006) died from drugs given with the intention to hasten death [84].

15.10 Severe Asphyxia

Stillborn infants do not breathe and show no other evidence of life (such as beating of the heart, pulsation of the umbilical cord, definite movement of voluntary muscles). If the infant shows signs of maceration, the infant has died at least 12 hours before birth. A fresh stillborn infant may have died hours or minutes before birth. If the fetal heartbeat was heard shortly before birth or fetal heartbeat was not monitored, resuscitation should be started.

Four studies on the outcome of infants with severe asphyxia defined by Apgar score 0 at 10 minutes following birth demonstrated that 69–98% died and all surviving infants developed severe (89–100%) or moderate neurodevelopmental disability [85]. This is why some justify cessation of resuscitation after 10 minutes [81], unless resuscitation was not adequate. If a baby develops signs of severe hypoxic-ischemic encephalopathy with poor long-term prognosis, a decision (jointly made by the parents and physicians) to withdraw intensive care is justified by some authors [78].

15.11 Malformations

Malformation may justify withholding of postnatal resuscitation if the malformation is not compatible with life or if there is a high risk of death or if therapy burden is not bearable. The

Table 15.4 Lethal malformations (according to the US American Birth Defect Center)

– Trisomy 13
– Trisomy 18
– Anencephaly
– Acranium
– Bilateral renal agenesis (Potter syndrome)
– Bart's hydrops fetalis

harm-to-benefit ratio (i.e., pain and suffering from early interventions in relation to the probable outcome) may be considered in the estimation of the best interests of the infant. Table 15.4 lists lethal malformations.

Termination of pregnancy for fetal malformation is liberally implemented in many countries. After birth the best interests of a viable infants with malformations have to be judged individually for each infant, each malformation and the probable long-term sequelae of the malformation. After birth, treatment decisions are primarily based on the best interests of the infant (and not primarily on the authority of the mother). This sudden change in authority is difficult to understand for many parents. Infants with Down syndrome pose particular dilemma for parents and physicians. On the one hand, the major purpose of prenatal screening is to detect trisomy 21 and to terminate pregnancy if the fetus suffers from Down syndrome, on the other hand there is general agreement that the diagnosis of Down syndrome is no indication for withholding resuscitation even if the infant suffers from severe cardiac or other malformations [86].

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16

Basic Approach to the Care of Extremely Low Birth Weight Infants: an Outline

Costantino Romagnoli and Fabio Mosca

First hours of life of extremely low birth weight (ELBW) infants are crucial. Several problems can arise in this time:

- at the first hour of life
  - resuscitation
  - thermoregulation
  - cardio-respiratory stabilization
- on admission to Neonatal Intensive Care Unit (NICU)
  - vascular access
  - skin
  - respiratory
  - cardiovascular
  - neurologic
  - fluid, electrolyte and nutrition
  - infections
- special issues
  - hearing impairment
  - retinopathy of prematurity
  - osteopenia
  - neurodevelopment.

16.1 Delivery Room and the First Hour of Life

At birth, careful attention to the maintainance of body temperature is essential. The most effective interventions seem to be gentle drying and occlusive wraps (polyethylene wraps or polyurethane bags), associated with hats. In spite of these measures, low temperatures are common on admission and surveillance of temperatures in the delivery room and during transport should be identified as quality improvement initiatives [1].

ELBW infants need some form of assisted ventilation in the delivery room in order to achieve adequate lung expansion. It is important to avoid hyperdistension (using low inspiratory pressures) and to maintain an adequate lung volume (using positive end-expiratory pressure). Best practice for the ventilation of newborn babies it is still matter of some debate. The use of continuous positive airway pressure (CPAP) may be a practice that reduces the use of mechanical ventilation [2].

Careful monitoring of heart rate, oxygen saturation and pCO_2, as soon after birth as practicable, is recommended [3].

Adequate vascular access is important for ELBW infants. Catheterization of umbilical vessels is frequently used for the administration of fluids and drugs.

16.2 Admission to the NICU

On admission to the NICU body temperature and body weight should be measured to correct or to prevent hypothermia and to optimize fluid management.

16.2.1 Vascular Access

Sick ELBW infants need close monitoring of arterial blood pressure and gases, serum glucose and electrolytes. Insertion of an umbilical and/or arterial catheter is useful to avoid pain and discomfort. Arterial lines should be removed as soon as they are no longer necessary and a percutaneous central venous catheter should be dedicated to infusion of parenteral nutrition (PN). Often these infants require more than one central line and the use of a double lumen umbilical venous catheter may be useful for the administration of fluid, drugs and blood products.

16.2.2 Skin

Just after birth, the skin of ELBW infants is poorly effective as an epidermal barrier. Disturbances in temperature regulation and water balance, as well as an increased risk of infection, occur because of poor epidermal barrier function and the excessive use of adhesives. The routine prophylactic use of emollients is not recommended.

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16.2.3 Respiratory

The goals of assisted ventilation should be: adequate oxygenation and ventilation avoiding barotrauma (due to high inspiratory pressures), stretching and rupture of the lung structures (due to volutrauma caused by high tidal volumes), and cyclical opening and closure of the closed alveolar zones (atelectrauma, caused by low positive end expiratory pressure).

In many centers, high frequency oscillatory ventilation (HFOV) is used as the initial ventilation strategy for the treatment of ELBW infants with acute pulmonary insufficiency in order to reduce the incidence of both early (e.g., air leaks) and late (e.g., bronchopulmonary dysplasia [BPD]) ventilatory complications [4]. Others prefer conventional pressure-cycled ventilation, synchronized intermittent mandatory ventilation (SIMV); hybrid forms of mechanical ventilation such as volume guarantee (VG), pressure regulated volume control (PRVC), and volume assured pressure support (VAPS) may be also used [5].

The optimal time for the administation of surfactant remains uncertain. It seems reasonable to administer surfactant as soon as oxygen requirements increase after birth. The timing of extubation is difficult because these infants are prone to severe apnea and frequent reintubations. Even if early extubation to nasal CPAP is possible for some because of methylxanthine administration (caffeine or theophylline), some require prolonged ventilation because of recurrent apnea or changes in lung dynamics (e.g., BPD), which may need different ventilatory strategies.

BPD is a major problem for these babies. Its frequency is variable.

16.2.4 Cardiovascular

The two major cardiovascular problems for ELBW infants are patent ductus arteriosus (PDA) and hypotension.

About 50% of ELBW infants show the effects of a hemodynamically significant PDA (left-to-right shunt) and the majority require pharmacological treatment with indomethacin or ibuprofen to close the ductus.

Although prophylaxis seems to be more efficacious than treatment for these infants, current best practice suggests a policy based on clinical and echographic evaluation of the ductus [6].

If clinically significant, or if the PDA fails to respond to pharmacological treatment, or if indomethacin or ibuprofen are contraindicated (about 20% of ELBW infants), surgical ligation should not be delayed.

When hypotension occurs in presence of hypovolemic shock volume replacement is required, while the use of plasma expanders and/or vasopressor drugs (dopamine or dobutamine) should be evaluated on the basis of infants attributes or blood arterial pressure and skin perfusion.

16.2.5 Neurologic

A cranial ultrasound scan should be done shortly after admission to NICU to document eventual developmental abnormalities. Careful monitoring using cranial ultrasound scans should be performed to diagnose both intraventricular hemorrhage (IVH) and its serious complications and periventricular echogenicities which are the antecedent of white matter injury (WMI) and of neurodevelopmental impairment. Although there is no question that magnetic resonance imaging (MRI) is superior to ultrasound for prognosis, ultrasound can give important early information and can be used to document the evolution of lesions, and is an important tool, particularly in many places where MRI is not available.

16.2.6 Fluids, Electrolytes and Nutrition

After birth, water and electrolytes should be supplied to ELBW infants to avoid dehydration and hypertonicity. There should be close monitoring of body weight (every 12–24 hours), urine output and osmolarity (every 12–24 hours) and plasma electrolytes (mainly sodium and potassium). Metabolic acidosis may become a problem with the progression of PN and its correction is still debated.

ELBW infants show limited nutritional reserves and immaturity of nutrient absorption in the face of high nutritional demands. For this reason, many ELBW infants experience postnatal growth retardation in spite of total PN and early enteral feeding. The main objectives of nutritional support are to maintain body stores and to assure adequate postnatal growth, avoiding metabolic complications and side effects.

ELBW infants should receive glucose and amino acid infusions soon after birth to maintain normoglycemia and protein levels. From the second day of life, lipids and electrolytes may be administered. Total PN should be associated with early enteral feeding to limit complications associated with PN, mainly cholestasis and sepsis.

Structural and functional immaturity of the gastrointestinal tract may limit early enteral feedings. Minimal enteral feeding (trophic feeding at 10–20 mL/kg/day) should be started as soon as possible after achieving clinical stability to stimulate normal functional development of the gastrointestinal tract. Its progression will be determined by feeding tolerance, which is normally reduced in ELBW infants. Feeding strategies depend on the experience of caregivers and should be aimed at the establishment of enteral feeding, avoiding feeding intolerance and necrotizing enterocolitis. Enteral volume (< 10 mL/kg/day) is increased gradually with close monitoring for abdominal distension, vomiting or increased volume of gastric aspirates, especially if bile-stained. When feeding intolerance is diagnosed, enteral feeding should be reduced or stopped. Breast milk is preferred to premature formulae for both trophic and enteral feeding, but when it is insufficient to

achieve normal postnatal growth, milk fortifiers can be added. Multicomponent fortification of human milk is associated with short-term improvements in weight gain, linear and head growth, but without evidence of long-term benefit.

16.2.7 Infections

Infection is a major cause of mortality and morbidity for ELBW infants. The incidence of early-onset sepsis (EOS) (<72 hours) is less than 5% with a mortality approaching 50%, mainly for ELBW infants born after premature rupture of membranes and/or chorioamnionitis. Prenatal treatment of chorioamnionitis and prevention of group B streptococcal infection has changed the distribution of pathogens of EOS with an increasing rate of gram-negative bacterial sepsis [7]. Taking a careful prenatal history, timely diagnosis (based on clinical signs, microbiological and biochemical tests) and therapy (empirical use of antibiotics) are important for the prevention and threatment of EOS and its complications.

Late-onset sepsis (LOS) affects 25 to 50% of ELBW infants and the mortality rate varies from 20 to 80%, depending on the causal pathogen. The main risk factors for LOS are the use of central lines, mechanical ventilation and parenteral nutrition. The clinical diagnosis of LOS is made more difficult in ELBW infants because of nonspecific clinical features and a lack of sensitive diagnostic tests. Thus, many of these infants receives several courses of antibiotics and antifungals during their hospital stay. Careful monitoring of a neonatal unit can help guide empirical antibiotic therapy and to limit antibiotic resistance [8]. Prophylactic intravenous administration of polyclonal immunoglobulins has been shown to reduce LOS, but was not associated with a reduction in other important outcomes or length of hospital stay and has not been widely adopted. Future use of monoclonal gamma-globulins for specific pathogens and of proteomics for diagnosis may offer new preventative and diagnostic opportunities [9].

16.2.8 Hematologic

Anemia is a frequent event for ELBW infants due to unavoidable multiple blood sampling, blood loss as a result of hemorrhage or hemolysis, rapid growth and low iron stores. Limitation of blood tests and the early administration of erythropoietin with iron supplementation may reduce the need for blood transfusions. Deficiency of vitamin K-dependent coagulation factors associated with low platelet count predispose ELBW infants to hemorrhage and should be promptly treated. Rarely disseminated intravascular coagulation may occur. All ELBW infants require phototherapy treatment for jaundice.

16.3 Special Issues

ELBW are also at high risk of hearing impairment because of illness and the use of ototoxic drugs, and require hearing screening in the neonatal period. Retinopathy of prematurity (ROP) is a special morbidity in ELBW infants.

Osteopenia occurs as a complication due to inadequate mineral intake, mainly phosphate and calcium. Its incidence increased when ELBW infants were feeded exclusively with human milk. Mineral support by total parenteral nutrition associated with preterm formulas or with fortified human milk have made osteopenia relatively unusual.

Recent observations suggest that ELBW infants are particularly vulnerable to external noxious stimuli: noise, light, pain, movements, frequent disturbances and manipulations. Neonatologists should pay particular attention to these problems and need to involve parents in individualized developmental care, practices to minimize stress and to optimize the neurodevelopment of each infant [10].

Finally, follow-up programs until adulthood have shown that about 20–30% of ELBW infants may have reduced growth patterns, severe neurological and physiological problems, neurosensory involvement, behavior and learning impairment.

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17

The Process of Decision-Making

Endla K. Anday and Maria Delivoria-Papadopoulos

When one prevents one's emotions from overtaking one's rationality it is called reason.
When one prevents one's rationality from overtaking one's emotions it is called compassion.
When one can do both it is called wisdom.
Ancient Chinese saying

17.1 Introduction

In the past 40 years, tremendous progress has occurred in the care of the neonatal patient. An in-depth understanding of fetal and neonatal physiology and the molecular basis of pathological processes, concurrent with advancements in biomedical technology, have impacted favorably upon the survival of the newborn. However, extremely premature infants at the threshold of viability, infants diagnosed with potentially lethal congenital malformations, including chromosomal abnormalities, and neonates suffering from profound intrapartum and postnatal insults present difficult moral, ethical, cultural and social challenges for both families and caregivers in regard to providing or withholding life supportive measures. The decision to withhold or terminate treatment for an infant is based on defining the issues central to this process, including: the values and rights of the infant as an individual, the values of the family and the medical caregivers and the cultural influences and societal values that shape the moral arguments that affect one's judgment. Part of the challenge resides in the complexity of defining *who is* or *should be* responsible for making difficult decisions resulting in the death of a newborn since fundamentally this type of decision is at odds with human nature even in situations where the end of life would mean no further suffering for the infant.

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The goal of this chapter is to consider some of the issues confronting the clinician charged with practicing the art of medicine in the context of a certain degree of medical uncertainty and the implications for medical decision-making. In-depth discussions of ethical problems and guidelines for the care of the extremely low birth weight infant (ELBWI) will be covered in other chapters of this book.

17.2 The Neuroscience of Decision-Making

Viner has stated that "Human behavior, in general is not under the constant and detailed guidance of careful and accurate hedonic calculations, but is the product of an unstable and irrational complex of reflex actions, impulses, instincts, habits, customs, fashion, and hysteria" [1]. Clearly, one would hope and expect that Viner's perspective of human behavior is not relevant to the process involved in making decisions to provide or withhold treatment for infants. Cognitive neuroscience supports that a variety of processes are essential to integrate novel incoming stimuli for decision-making in situations of uncertainty such as those pertaining to life-sustaining medical treatment. When considering options about incoming information, the key individuals involved must demonstrate flexibility to account for various outcomes, constantly monitor incoming information as it relates to the ultimate goal-state or decision and evaluate the risk-benefit ratio for various decision making options [2]. But medical decisions are not simply the rational calculation of benefit versus burden. The individuals involved often consider past experience and values as well as future outcomes of their decisions as part of the basis for that choice. As such, a certain degree of individuality is defined that contributes to decision-making, particularly when one considers the emotional influences on this process. In humans, emotions influence decision-making. Internal cues and external contextual information can positively or negatively impact the emotional state surrounding the decision-making process. The emotional evaluation of an action's consequences

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guide the individual who must be able to provide a basis for making certain choices [3]. Social neuroscience supports that the neuroanatomical substrate involved in complex decision-making in humans involves the integration of contextual social knowledge represented as event knowledge in the prefrontal cortex (PFC); social semantic knowledge, stored in the anterior and posterior temporal cortex; and motivational and basic emotional states, which depend on cortical-limbic circuits [4, 5]. While certain perceptual and emotional abilities have been shown to be shared by humans and other animals, morality and moral decision-making is a product of evolutionary pressures specific to the human species that have shaped social cognitive and motivational mechanisms into uniquely human forms of experience and behavior [6]. Experiments using functional magnetic resonance imaging (MRI) in humans have identified that decision-making involving intelligence and complex resolution activates the ventral and medial aspect of the prefrontal cortex (VMPFC) [7]. Activation of the VMPFC appears to signify that wisdom required in the decision-making process comes from categorizing and organizing past experiences from many situations, and involves options for actions, deciding what actions to take and factual emotional outcomes [8].

Thus, the evolution of the human PFC is intimately related to the emergence of human morality, allowing for the motivational mechanisms to be integrated with an exceptional power to predict outcomes from a large repository of experiential wisdom. Coupled to semantic knowledge, cultural and situational factors become integrated into the neural substrate to weigh the motivational relevance of different behavioral choices in social situations. Clearly then, through a complex interaction of biological and cultural factors, the human moral mind emerges and integrates cognition to emotion and motivation, forming the framework for decision-making.

17.3 Steps in Decision-Making

17.3.1 Recognize the Moral Dimension

From a philosophical approach, Neonatal Medicine has been practiced to provide specialized and intensive care measures aimed at improving the health and survival of premature and critically ill newborns. But how are these goals affected when the curative model is inadequate, such as with infants diagnosed with anencephaly or with the delivery of a 22 week gestational age infant; what do the goals become? And why is decision-making in this context so difficult? Consider the situation wherein prenatally a mother is diagnosed as carrying a fetus with severe potentially lethal congenital anomalies; the physician is put into a unique situation in medicine in which he/she contemplates withholding support in a human being *that the physician has never even seen*! Or contemplate the situation of the 23 week gestational age infant with bilateral Grade III intracranial bleeds and pneumoperitoneum trapped in NICU biomedical technology, hanging on to life by a thread and without reasonable expectation for improvement without incurring unnecessary pain and suffering; when is enough really enough? Compound the dilemma with both parental and physicians' attitudes toward life-saving technology and death, which have been shown in numerous studies to vary with race and ethnicity, and are thought to result from complex interactions of factors including: access to medical care, communication styles, family involvement in decision-making, trust in physicians and religious background [9].

Each healthcare dilemma reminds caregivers of their limitations, including uncertainty, the human predicament, lack of knowledge, and decision-making abilities. All of these are tempered by the moral constraints under which we act. These are the difficult situations which, based on individual social, cultural, ethical and moral differences, make it highly unlikely that we will all agree. Nevertheless, within the context of providing care to the high-risk neonate, the best interest of the child must be kept foremost in the minds of the caretakers and the professional staff and form the basis for decisions related to the care of that infant.

17.4 Recognize the Parties Involved

17.4.1 The Infant in His/Her Own Right as an Individual

The infant born at the threshold of viability or one who has potentially lethal congenital anomalies has no voice of its own; it depends on decisions made by a surrogate, most often its mother and/or family members to advocate on its behalf. Under ideal circumstances, prenatal diagnosis and counseling allows for effective communication between parents and physicians and a reasonable approach to initiating or withholding resuscitation can be achieved based on the prognosis and risk versus benefit of treatment. During this decision-making process, the value of the infant's life is being judged. However, there may be disagreements between the medical care providers and surrogates where, in the opinion of the provider, the infant's best interest is not being served and in which the surrogate's decision should be over-ridden [10]. In 2001, the Senate and House of Representatives of the United States Congress passed the "Born Alive Infant Protection Act". Specifically, the Act provides that any "member of the species *homo sapiens at any stage of development* that is expelled or extracted from the mother who then breathes or has a beating heart or movement of voluntary muscles is to be considered a person and granted full protection under the U.S. Constitution" [11]. The Act provides that even a fertilized ovum, embryo and fetus of any gestation are to be considered co-equal with regard to protection from harm. While it was widely understood to be an anti-abortion legislation, the Act

altered the definition of the words "person", "human being", "child", and "individual". Although the law did not impose a new standard of medical care upon physicians, nor change existing law, it altered the physician norm of deferring to parental discretion regarding the initiation or discontinuation of medical treatment for the extremely premature infant. However, the American Academy of Pediatrics (AAP) and the Neonatal Resuscitation Program Steering Committee issued an opinion that it would not affect day-to-day neonatal medical practice and standards. In fact, the Committee clearly stated that "decisions about withholding or discontinuing medical treatment that is considered futile may be considered by the medical care providers in conjunction with the parents… Those newly born infants who are deemed appropriate to not resuscitate or to have medical support withdrawn should be treated with dignity and respect, and provided with 'comfort care' measures" [12].

Consider the Baby Doe Law, passed in 1984 in the United States that sets forth specific guidelines for the treatment of seriously ill or disabled newborns and dictates what must be done for a child regardless of the wishes of the parents [13]. Withholding treatment is considered neglectful unless an infant is irreversibly comatose or the treatment is futile in terms of the infant's survival, but "quality of life" is not a reason for withholding medical care. As a result of this law, hotlines were set up for anonymous reporting of alleged abuse such as withholding of medical care to seriously ill newborns leading to intrusions into the hospitals that were disruptive and ultimately found unwarranted. This Act was overturned in the Federal Courts in 1987.

Two laws, each illustrating potential conflicts between two or more values and the rights of the child as a distinct entity contrasted to the rights of parental decision-making as the surrogate for the infant. Even more intriguing is the observation that, in spite of legal and ethical standards requiring resuscitation when it is considered to be in a patient's best interest, the newborn infant and particularly the preterm baby is devalued in comparison to older patients with comparable survival and morbidity outcomes [14]. In a questionnaire describing eight neurologically incompetent patients with potentially equivalent adverse neurological outcomes who required resuscitation (24 week preterm, term infant, 2 month old infant, 7 year old child, 14 year old, 35 year old, 50 year old patient and an 80 year old subject), respondents were asked whether they would comply with the families' wishes if resuscitation was refused. The families' refusal of resuscitation was accepted most frequently for the 80 year old subject and the 24 week premature infant, even though the majority of the respondents thought that resuscitation was indicated for the 24 week infant but not the 80 year old patient. Since the decision was not made on the basis of outcome or what was perceived to be in the infant's best interest, there seems to be a different value placed on life at the extremes. While the root of this devaluation is not clear, it may be attributable to anthropologic, cultural, social and evolutionary factors.

17.4.2 Parental Decision-Making

Regardless of the concept of provision of care, it is essential that parents understand the implications of the nature of aggressive care in situations where they "want everything done" for their infant and physicians must be willing and prepared to recommend withdrawal of support in futile situations. Throughout this process, the infant must be afforded compassion and comfort. What is good for critically ill newborns and who determines this? The presence of numerous voices in deliberations about newborn patient care presses this question. What we as health care professionals think may be quite different from what the family or child might choose.

In the face of uncertainty and inability to predict outcome for an individual infant, there will usually be differences of opinion regarding outcome, viability and treatment futility. Those who bear the ultimate burden of the decision ought to have the major role in the decision making when outcomes are very uncertain. Simply put, once a physician has determined that an infant has no realistic expectation of survival based on the evidence at hand, parents should be informed and given the opportunity to withhold initiating treatment (e.g., prenatally diagnosed infant with trisomy 18 and complex congenital heart disease) or given the option of withdrawing the life-supporting interventions (e.g., 23 week gestational age ELBWI with bilateral Grade IV IVH and necrotizing enterocolitis totalis). When faced with such a difficult moral decision, parents often will look to the physician for advice and counseling. The Committee on Bioethics of the American Academy of Pediatrics has advocated for a shared decision-making model whereby the physician has an ethical duty to seek and respect information regarding parental preferences and values in exchange for providing the parent with medical information [15]. A nondirective approach to counseling parents is advocated to allow parents to choose a course of action based on their own personal values. While the transfer of information from physician to parent should be unbiased, the manner in which information is communicated may have a framing effect on the parental decision. The recent study by Haward et al [16] examined message framing and perinatal decisions and demonstrated that the manner in which parents received prognostic information, i.e., framed as mortality data versus survival data, affected their treatment preference independent of religiousness, parental status and beliefs regarding the sanctity of life [16]. In subtle ways, framing bias can inadvertently affect the process of parental decision-making at a time when decisions dependent on moral values that deal with the consequences of life and death occur in close proximity to physician counseling.

However, for some parents non-initiation of or withholding life-sustaining treatment is a psychologically and morally impossible choice; they may want treatment withdrawn or not initiated so their child does not suffer, but do not want the responsibility of making that decision because they do not want to give up hope. Under these circumstances, a "left unasked

but not unaddressed" approach may allow transition of aggressive, futile care to care that is based upon medical indications [17]. For example, instead of asking a parent if they want to initiate dopamine infusion for their infant with bilateral Grade IV intraventricular hemorrhage (IVH) and refractory hypotension, and giving them false hope, one can explain that everything reasonable has been done for their baby, that keeping their infant comfortable now and holding their baby is the best one can do at this time. In this manner, the parents are not tormented with decisions fraught with anguish, ambiguity, doubt and guilt. There comes a time when "there is no need to compound the suffering of the patient by prolonging it, nor that of the parents by insisting they must make a decision as fate has already decreed" [17].

17.4.3 The Physician's Role

At the time of delivery of an extremely premature infant or one with potentially lethal congenital anomalies, it may be difficult for a physician to predict for parents what the outcome will be: possibilities range from death soon after delivery to survival with severe disabilities to intact survival. Similarly, an infant might have an initial postnatal course that would favor a reasonable outcome, only to suffer a devastating event in the NICU where prognosis is poor. The physician is placed in a situation of weighing the benefits and burdens of ongoing support for the infant under conditions of medical uncertainty and communicating information in an effective manner to the parents, keeping in mind that the decision is in keeping with legal and organizational guidelines. The roles of authoritative statements, professional policies, and recommendations can be helpful in the decision-making process for the physician, however, most decisions are not based purely on a utilitarian approach.

During the course of refining the practice of neonatal medicine, a wealth of information relating to the care of the critically ill newborn has been accumulated, not only in the United States, but world-wide. A number of NICU guidelines and statements have been developed to establish norms and reference points in an effort to assist physicians in day-to-day management of difficult situations including withholding or terminating life-sustaining therapy, but these may at times be of limited use in the discussion of risks, benefits and other options with the parents and families [18, 19]. Although objective outcome data are available to many clinical populations, for a given infant and in many situations, the chance of a certain outcome may be either 0 or 100% [20, 21]. The recommendations by the International Liaison Committee on Resuscitation for guidelines on neonatal resuscitation supports the "do-not-resuscitate" order on evidence-based justification for the extremely low birth weight infant < 23 weeks and < 400 grams and confirmed trisomy 13, 16 and 18 [22]. However, upon review of incorrect information, diagnosis or prognosis about a specific situation, the guidelines provide for the opportunity of a trial of therapy with the option of subsequent withdrawal of support. The Groningen protocol considered euthanasia under strict protocol in situations where there was severe and sustained suffering without relief [23]. Newborns are classified into three categories depending on the severity of their condition: Group 1 is infants who are expected to die in spite of invasive medical care and are actively removed from support to be held by the parent(s); Group 2 are infants who survive birth but are expected to have poor quality of life and who cannot survive without the assistance of invasive technology; and Group 3 are patients who could eventually survive without technology but who suffer severe, sustained pain and a life without hope of improvement. Of the three groups, Group 1 is least likely to present a dilemma. In Group 2 patients, quality of life issues form the basis for the decision-making and in this situation, it is critical to maintain the best interest of the child in consideration and involve the opinion of the parent(s). However, the decision should not rest solely with the parent; discontinuation of support must be in concert with the physician together with the parent(s). At all times, the infant should be kept comfortable and without pain. The last group presents the most difficult situation because it is a judgment call that death would be more humane than continuation of life. A passive approach to allowing "nature to take its course" and withhold creature comforts, such as nutrition, can lead to a period of severe suffering until death Alternatively, deliberate termination of life, or euthanasia with the use of medications to end the suffering is considered illegal. The recent article by Verhagen and Sauer [23] discussed the Groningen protocol in regard to strict reporting and review of each case of proposed active termination of life to ensure that acceptance of this practice will not allow erosion of moral norms where other undesirable qualities in newborns can be used as justification to end their lives.

Physicians and parents may have different interpretations of what is discussed and what decisions are made during prenatal counseling, in the delivery room and following the delivery of an infant. Studies support that it is the parents' perception that physicians do not always address values that parents consider important in the counseling they provide [24–26]. Specific parental values such as hope, spirituality and religion are often not incorporated in the discussions and may contribute to confusion about what has been discussed and what decisions have been made [27]. To ignore these questions is to fail to recognize the significant influence that these values and cultural influences have had in shaping individual professional lives and human interactions.

17.5 Summary

Ours is a global society in which one must be increasingly aware of the different value systems in multicultural societies across nations. While a deeper understanding of the cognitive

and brain mechanisms that guide human behavior is of general interest, the moral and ethical decisions that are made regarding withholding or withdrawing care to an infant provoke anxiety and guilt that come with responsibility for making difficult decisions. In the end, the goal of care for the newborn must be focused upon the best interests of the child. Parents generally should be considered the spokesperson for determining what is in the best interest of the child; therefore, the medical team should seek the opinions of the parent(s), both prior to and following delivery of a child identified as extremely high risk for adverse outcome.

A period of transition to extrauterine life, allowing for stabilization and evaluation of the infant to assess the degree of significant intervention and determine the likelihood of reversal of acute processes should be offered. When evidence-based guidelines are available as a frame of reference, one may choose to work within a set of guidelines; however, these must be individualized for each circumstance. Prognosis can change rapidly, from the time of birth, to the delivery room resuscitation; the likelihood of "reasonable outcome" should be reevaluated at each junction. Implicit in the decision-making is the assumption that parental counseling has occurred. Parental wishes regarding extent of intervention initially should almost always be honored until further determination by the medical team supports the futility of further care. In the absence of a reasonable expectation of steady improvement and in the presence of significant pain and suffering, withdrawal or withholding life-sustaining medical or surgical treatment is a decision that takes the best interest of the baby in consideration.

In the Hippocratic Corpus, medicine is defined as having three roles: "To do away with the suffering of the sick, to lessen the violence of their diseases, and to refuse to treat those overmastered by their diseases, realizing that in such cases medicine is powerless" [28]. In the end, perhaps decision-making may best be summarized by the Jesuit ethicist, Father John Paris, "The best one can do is to make a human judgment based on probabilities" (personal communication).

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18

Follow-up Outcomes of High Risk Infants

Maureen Hack and Deanne Wilson-Costello

18.1 Introduction

Advances in obstetric and neonatal care following the introduction of methods of neonatal intensive care in the 1960s resulted in a progressive improvement in the survival of high-risk neonates until the mid to late 1990s [1]. However, the survival of acutely ill preterm infants who previously would have died together with iatrogenic factors, such as the use of postnatal steroids, resulted in an increase in neurodevelopomental impairments during the 1990s [2]. Since 2000, the rates of mortality of preterm infants have remained fairly stable and there has been an encouraging decrease in the rates of cerebral palsy. This has contributed to an overall decrease in neurodevelopmental impairments, especially for extremely preterm infants (Fig. 18.1) [3–5].

Follow-up of high risk newborns is important for many reasons. Firstly from a clinical care perspective, there is a need to provide ongoing surveillance of the growth and development of the survivors of neonatal intensive care and to provide specialized care for those who develop complications of prematurity such as bronchopulmonary dysplasia or perinatal brain injury with resultant neurosensory inpairments. Surveillance of the outcomes of neonatal intensive care is also necessary in order for each perinatal center to be able to monitor the overall quality of care in their neonatal intensive care nursery as well as the specific effects of new therapeutic interventions on outcomes. Such information is also necessary in order to be able to council parents as to the potential outcomes of their infants. These may be birth weight or gestational age specific outcomes or the outcomes of specific neonatal complications, for example periventricular leucomalacia or severe periventricular hemorrhage. From a research perspective, follow-up of infants until at least into the second year of life has become an integral part of randomized controlled trials of perinatal intervention and of cohort studies which monitor changes in outcomes over time. Outcomes examined usually include measures of growth, neurosensory impairments including rates of cerebral palsy, blindness and deafness and cognitive function, as well as specific health care needs. Cerebral palsy is the most common of the neurosensory deficits among preterm children. The outcomes of preterm children are usually reported as rates of neurosensory deficits and developmental outcomes, which include early childhood cognitive assessments such as the Bayley Scales of Infant Development or intelligence tests at school age [6].

The organization of follow-up programs of high risk newborns depends on the health care system of the specific region. In most countries the majority of clinical follow-up care of neonatal intensive care graduates is undertaken by pediatricians or the family physician, with referral to subspecialists for specific complications. National data bases, when they exist, may be used in such instances to provide data on the outcomes. Academic tertiary care centers, however, usually organize follow-up programs as an integral part of their neonatal care intensive care unit. These follow-up programs serve not only to provide surveilance of growth and development of selected groups of survivors but also coordinate and undertake the follow-up of subjects who have participated in randomized controlled research trials. Such programs also serve to educate medical students, pediatric residents and neonatal fellowship trainees in the potential outcomes of the children they have treated in the intensive care unit and in the essential skills required to assess growth and development of high risk neonates. A minimal requirement for the clinical monitoring of outcomes is a periodic assessment of growth and neurosensory development during the first 2 years of life. The ideal is a comprehensive program including all aspects of care, including well-baby care, evaluation of outcome, social and educational intervention, and therapy when needed.

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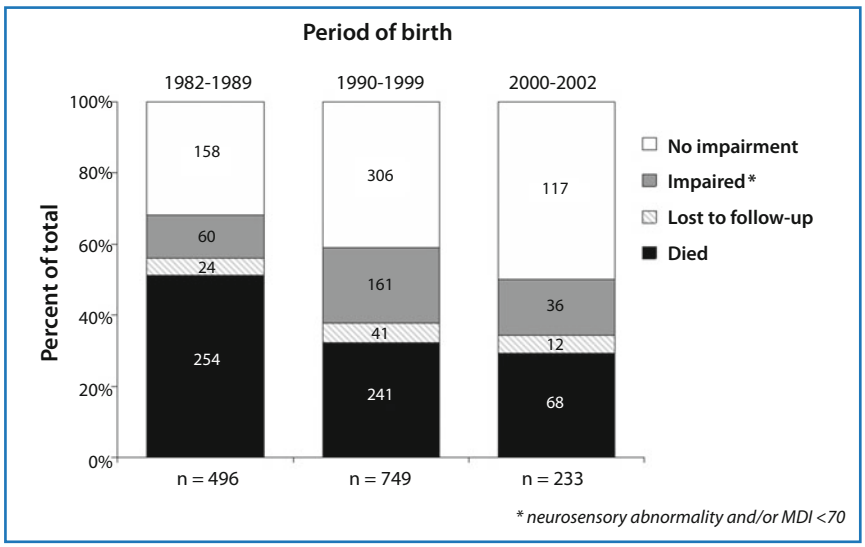

Fig. 18.1 Comparison of death and survival with and without neurodevelopmental impairment at 20 months corrected age for 500–999 gram birth weight infants born during 3 periods: 1982–1989; 1990–1999 and 2000–2002. Impaired: neurosensory abnormality and/or MDI <70 (MDI Mental Developmental Index). Reproduced from [5], with permission

Children at highest risk for poor health and developmental outcomes include those born at extremely low birth weight (<1 kg) and gestational age (<28 weeks) who have high rates of neonatal complications such as bronchopulmonary dysplasia and cerebral hemorrhage, infarction or periventricular leukomalacia (Table 18.1). However, there is a gestational age gradient in outcomes and even late preterm infants born at 34–36 weeks gestation have higher rates of health and developmental problems than term born children. Problems that predispose to poor outcomes among term born children include severe birth depression, severe respiratory failure including persistant fetal circulation, meningitis, severe intrauterine growth failure, intrauterine infections, severe hypoglycemia and kernicterus following severe hyperbilirubinemia. The majority of these problems can also affect the preterm and late preterm infant.

Perinatal outcomes are influenced by the demographic and socioeconomic profile of the family, the incidence of extreme prematurity, a selective treatment or admission policy, the percentage of inborn patients at any center, and the rate of follow-up [7]. Intercenter differences in neonatal sequelae and outcome are well described [8, 9]. Regional results therefore reflect a more accurate picture of outcome because they include all infants born in an area. Other factors to be considered are the rate of loss of infants to follow-up, the neonatal and post-discharge death rate, the age at follow-up, and the method of follow-up (Table 18.2) [10]. Two years is the earliest age to get a fairly reliable assessment of neurodevelopmental outcome. Follow-up to school age allows for a more stable assessment of cognitive function and for the indentification of subtle neurologic and behavioral dysfunction. Other important measures at school age include tests of academic achievement (spelling, reading, math) and school failure or need for special education. Parent questionnaires may also provide information on the behavior and social functioning of the child, quality of life, special health care needs the child may have and impact on the family. Similar measures may be obtained during adolescence at which time the child can also be interviewed.

Table 18.1 Factors affecting outcome of the preterm infant *

- Birth weight <750 g or <25 weeks' gestation
- Periventricular hemorrhage (grades III and IV) or infarction
- Periventricular leukomalacia
- Persistent ventricular dilation
- Neonatal seizures
- Bronchopulmonary dysplasia
- Neonatal meningitis
- Subnormal head circumference at discharge
- Poverty and parental deprivation
- Coexisting congenital malformation

* Adapted from Wilson-Costello D, Hack M. Follow up of high risk neonates (2006) In: Martin RJ, Fanaroff AA, Walsh MC (eds) Neonatal-perinatal medicine diseases of the fetus and infant, Vol 8, Mosby.

18.2 Health and Medical Problems

Respiratory problems especially during the first year of life, during the winter months and among infants who suffered from bronchopulmonary dysplasia, include frequent wheezing, upper and lower respiratory infections and a susceptibility to the respiratory syncitial virus. These may result in increased rates of rehospitalization. The respiratory problems diminish during the second year of life although asthma and airway reactivity may persist.

Children with neurologic sequelae such as cerebral palsy and hydrocephalus may also require rehospitalization for shunt complications, surgical correction of spasticity and eye surgery for strabismus.

Table 18.2 Factors to consider when evaluating long-term outcome studies

1. *Rate of survival*
2. *Type of study*
   Longitudinal
   Cross-sectional
   Controlled trial or intervention pharmacotherapeutic (e.g., surfactant) or educational enrichment
   Examination of specific risk factors (e.g., brain hemorrhage, chronic lung disease, etc.)
3. *Study sample*
   Hospital-based or regional
4. *Sample characteristics*
   Birth weight and gestational age range
   Rates of intrauterine complications
   – Intrauterine growth failure
   – Intrauterine infections
   – Congenital malformations
   Perinatal morbidity (e.g., periventricular hemorrhage, chronic lung disease
   Sociodemographic descriptors (e.g., sex, race, marital status, education)
   Parental substance abuse
   Post-discharge medical care and morbidity
   Post-discharge intervention or enrichment programs
5. *Suitability of comparison (control) groups*
6. *Rate of loss of follow-up*
7. *Duration of follow-up*
   Infancy
   Early childhood
   School age
   Adult outcome
8. *Correction for preterm birth*
9. *Outcomes measured*
   Growth
   Health status
   – Illness (e.g., respiratory, rehospitalization)
   – Post-discharge death rates
   Neurological status
   – Cerebral palsy
   – Blindness
   – Deafness
   Neuropsychiatric outcomes
   – Intelligence
   – Speech and language
   – Psychomotor
   – Memory
   – Attention
   – Executive function
   Academic achievement
   School performance
   – Failure
   – Need for special education
   Behavior
   Social competence
   Functional abilities
   Quality of life
   Impact/effect on family
   Socioeconomic
   – Cost of hospital stay
   – Cost of education

18.2.1 Growth

Intrauterine growth failure occurs commonly among preterm infants and may be associated with maternal complications such as preeclampsia or fetal conditions such as multiple birth. Studies of the postnatal growth of extremely low birth weight and gestational age children born in the 1990s, who were born approprite for gestational age, revealed that the majority developed growth failure during the neonatal period. This was related to a conservative approach to early parenteral and oral nutrition and to the development of chronic conditions of prematurity including necrotizing enterocolitis, bronchopulmonary dysplasia and repeat episodes of late onset sepsis. Poor growth among infants with bronchopulmonary dysplasia and those with neurologic impairment may occur both during the neonatal and post-discharge periods and be associated with swallowing dysfunction, oral aversion and gastroesophageal reflux. A more aggressive approach to early parenteral and oral nutrition together with increased caloric formulae during the neonatal period and after discharge has improved the growth of preterm children during infancy, however, whether this advantage persists later in childhood is as yet unknown [11].

Growth, weight, length and head circumference should be measured serially during the first 2 years of life to assess both catch-up growth in infants who suffered previous intrauterine or neonatal growth failure and to monitor for fall off in growth which may occur following discharge from the intensive care unit, especially in neurologically impaired infants and those who suffered from bronchopulmonary dysplasia. Fluctuations in growth may also occur in healthy children although this needs to be closely monitored. The majortiy of catch-up growth occurs during the first 2 years of life, however, catch-up growth may occur later during childhood and during the adolescent growth spurt. The genetic effects of parental size on length/height usually influence the growth of children after the first year of life [12].

Growth of the child should ideally be compared to local growth norms. Alternatively, the Center for Disease Control (CDC) growth norms may be used [13] or the World Health Organization (WHO) growth norms [14]. Weight during infancy and early chidhood should be measured with the child undressed. Recumbant length should be measured using a pediatric stadiometer and the maximal frontal occipital head circumference with a non stretch measuring tape. Other measures of growth may include the Body Mass Index after the age of 2 years.

18.2.2 Neurodevelopmental Outcomes

The neurologic examination during infancy is largely based on changes in muscle tone that occur during the first year of life. The examination developed by Amiel-Tison, which is

commonly used, measures the progressive increase in active muscle tone (head control, back support, sitting, standing, walking) together with the concomitant decrease in passive muscle tone. This also documents visual and auditory responses and some primitive reflexes. This method gives a qualitative assessment of neurologic integrity, which is defined as normal, suspect, or abnormal. A conventional neurologic examination should be performed thereafter, together with the Amiel-Tison method for early childhood [15, 16].

18.2.3 Transient Neurological Abnormalities

Transient abnormalities of muscle tone during the the first year of life include abnormalities of muscle tone such as hypotonia or hypertonia (occurring as poor head control at 40 weeks' post-conceptional age, poor back support at 4–8 months, or a slight increase in muscle tone of the upper extremities). The increase in muscle tone is often termed dystonia. Although transient neurologic abnormallities may resolve during the second year of life, they may indicate later subtle neurologic dysfunction which is not measurable during early childhood, or school learning difficulties [17].

18.2.4 Major Neurologic Sequelae

Major neurologic disability is usually classified as cerebral palsy, hydrocephalus (with or without accompanying cerebral palsy or sensory deficits), blindness (usually caused by retinopathy of prematurity), seizures, or deafness. The type of cerebral palsy mostly seen among preterm children pertains to the spastic type which includes bilateral (diplegia, quadruplegia) or unilateral (hemiplegia) spasticity. The athetotic and dyskinetic types of cerebral palsy are usually seen among term born children.

Cerebral palsy is the best clinical marker available to monitor the effects of perinatal and neonatal complications and therapies on the long-term outcomes of preterm children, as measures of cognition and intelligence are highy influenced by sociodemographic, environmental and genetic family effects. Until the 1990s cerebral palsy was defined as a non-progressive disorder of movement, posture or both [18]. When function was considered, cerebral palsy was defined as mild, with no loss of function and independent walking; moderate with functional disabilities requiring assistance for walking with aids or walkers; and severe, nonambulatory, requiring a wheelchair. Cerebral palsy was alternatively labeled "disabling" or "nondisabling" [19].

In 1997, Palisano et al [20] developed a reliable functional classification system to quantitatively measure gross motor function in children with cerebral palsy. This Gross Motor Function Classification System (GMFCS) defines motor function on the basis of self-initiated movement with particular emphasis on sitting, walking and mobility using a five level classification system. Distinctions are based on functional limitations, the need for hand-held mobility devices such as walkers, crutches or wheeled mobility, and to a much lesser extent, quality of movement. Since classification of motor function is dependent on age, separate descriptions are applied over a variety of age ranges. An example of the classification system used for toddlers is presented in Fig. 18.2. In 2004, an international workshop on the definition and classification of cerebral palsy proposed inclusion not

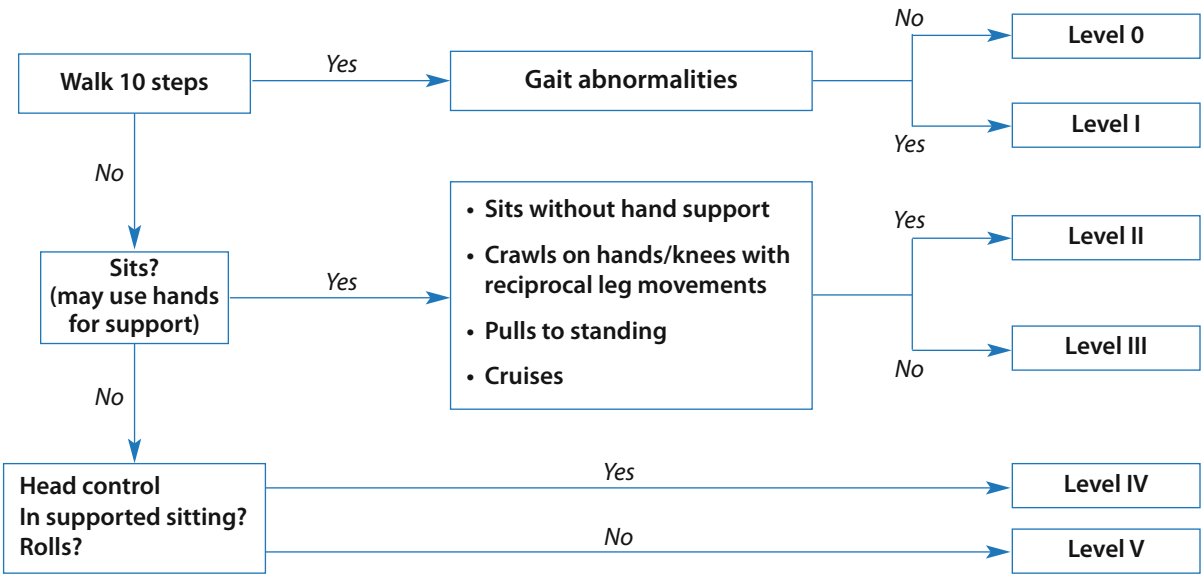

Fig. 18.2 Gross motor classification system for toddlers. Data from [20]

only of motor disorders but the functional abilites of the child and other associated deficits which may coexist including seizures and cognitive, perceptual, sensory (visual and hearing), and behavioral impairments [21]. The classification also includes anatomic and radiologic findings and causation and timing of the lesion. Its use should improve the classification of children with cerebral palsy and facilitate studies of trends in the rates of cerebral palsy and its correlates.

When examining rates of cerebral palsy in a population it is important to differentiate between the rates per live births, that is the total population at risk, including those who die, as compared to the rates of survivors only. Although many studies report rates of cerebral palsy at age 18–24 months, a definitive diagnosis should not be made prior to 3 years of age and preferably 5 years. This is due to the fact that some cases diagnosed at age 2–3 years may not be considered as cerebral palsy at age 5 years [22].

18.3 Timing of Clinical Follow-up Visits

For clinical purposes, the initial follow-up visit should be 7–10 days after discharge from the neonatal nursery. This is important in order to evaluate how the child is adapting to the home environment. A clinic visit at about 4–6 months of corrected age is important to document problems of inadequate catch-up growth and severe neurologic abnormality that might require intervention or occupational and physical therapy. Eight to twelve months of corrected age is a good time to identify signs of developing cerebral palsy or other neurologic abnormalities. For research purposes 18–24 months of age is the earliest age to obtain a fairly reliable assessment of both neurologic outcome and early cognitive function. By 3 years of age, other measures of cognitive function can be performed that better validate the child's mental abilities. Language is well measurable at this age. From 4 years of age more subtle neurologic, visuomotor, and behavioral difficulties are measurable.

18.3.1 Developmental Testing

The Bayley Scales of Infant Development are the most commonly used tools for monitoring early cognitive and motor development for high risk infants. The scales were developed for children aged one month to 3.5 years of age. The first and second editions of the Bayley Scales (BSID and BSID-II) were divided into three subtests or scales: mental, motor and behavior and provided a Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) with a mean of 100 and a standard deviation of 15 [23, 24]. The 1992 revision yielded lower scores for survivors than those described for children born during the 1980s and early 1990s and tested with the original BSID. The MDI and PDI were 12 and 7 points lower respectively than the original version of the Bayley Scales. In 2006, the most recent iteration of the Bayley Scales, the third edition (BSID III) was introduced [25]. This revision includes five major areas of development for early childhood assessment from birth through 3 years of age. The five scales include cognitive, language, motor, social-emotional and adaptive behavior, the latter two in the form of parent questionnaires. Thus BSID III does not generate a "mental developmental index" but rather separate cognitive and language scores. The cognitive composite of the Bayley III is 6 points higher than the previous MDI and the motor composite is 8 points higher than that of BSID II [26]. These differences need to be considered when comparing outcomes of high risk children over time. Infant tests prior to 2 years of age measure simple cognitive functions. They cannot be considered as tests of intelligence and have poor predicitve validity for tests of intelligence at school age [27].

Parent questionnaires may also be used for developmental screening. The Ages and Stages Questionnaire is one such questionnaire that may be used as a screening tool for children age 3 months–5 years in order to identify developmental delays [28]. However, it does not give a quantitative assessment and thus cannot be used to quantify outcome in specific high-risk populations.

18.3.2 School Age Outcome

Measures at school age should include growth, a neurologic exam, gross and fine motor function, intelligence both verbal and non-verbal, neuropsychologic functioning including executive function, memory and learning and tests of academic achievement including reading spelling and math as well as measures of behavior.

For intelligence the Wechsler Scales (WPPSI-III and WISC-III) may be used with prekindergarten and school-age children. The WISC-III is probably the most commonly used instrument for school age assessment. The Kaufman Test was standardized on a 2 1/2 to 10 year old population and is less heavily weighted by verbal items than other tests. Although the mean IQ of preterm children falls within the low normal range, the rates of borderline (70–84 IQ) and subnormal (<70 IQ) are significantly higher when compared to term born children of normal birth weight.

A recent meta-analysis of the neurobehavioral outcomes of very preterm or very low birth weight children at school age reveals significant differences between preterm children and controls in academic achievement (including mathematics, reading and spelling), in executive function (verbal fluency, working memory and cognitive flexiblity and congitive flexibility) and in behavior, including poor attention, hyperactivity and internalizing symptoms, which includes withdrawn, anxious and depressed behavior. These school age problems are associated with the need for special education and poor social functioning [29].

18.4 Functional Limitations and Special Health Care Needs

Children with special health care needs are defined as those who have, or are at risk for having a physical, developmental, behavioral, or emotional condition and require health or related services of a type or amount beyond that required by children generally. In the United States this definition is used for the identification and planning of federal aid and services for children which thus includes many preterm children [30]. Due to their higher rates of medical, neurologic and developmental problems, extremely preterm children have many more functional limitations, compensatory dependency needs and require many more services above routine than term born children (Figs. 18.3 and 18.4). A parent questionnaire, the Questionnaire for Identifying Children with Chronic Conditions (QUICCC) was utilized for a study in Cleveland of <1 kg birth weight children born 1992–1995 [31, 32]. Results revealed that 64% of the children had one or more functional limitations, as compared to 20% of normal birth weight children. Functional limitations included limitations in activities of daily living such as physical and social activities and difficulties in feeding dressing and toileting; 48% had compensatory dependency needs which included need for special medications, special equipment to see, hear or walk and help or equipment to feed, dress, wash or toilet compared to 23% of normal birth weight children. Sixty five percent of the children required special services for chronic conditions, as compared to 27% of normal birth weight children. These included physical or occupational therapy, nursing care, rehospitalizations, and special education or extra help at school. These differences remained significant even when children with neurosensory impairments were excluded. However, with the exception of a small minority of children with severe disabilities, the majority of services pertain to special education services for school learning problems.

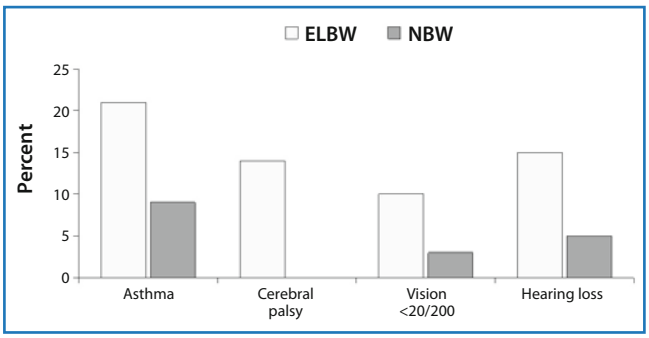

Fig. 18.3 Rates of asthma, cerebral palsy, vision < 20/200 and mild hearing loss (unilateral or bilateral hearing loss of more than 25 dB in at least 2 frequencies) of extremely low birth weight (ELBW, < 1kg birth weight compared to normal birth weight (NBW) children at age 8 years. Data from [32]

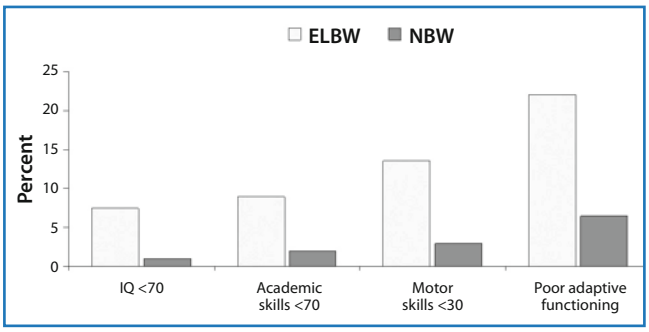

Fig. 18.4 Rates of subnormal IQ, academic skills, motor skills and poor adaptive functioning of extremely low birth weight infants (ELBW, <1kg birth weight) compared to normal birth weight (NBW) children at age 8 years. Data from [32]

18.5 Outcomes of Extremely Preterm Infants

Two European regional studies reported important information about the poor school age outcomes of extremely preterm children. The Epicure study of infants born between 20–25 weeks gestation in the United Kingdon and Ireland in 1995 revealed that at age 6 years 21% of children had cognitive disability defined as more than 2SD below the mean. However, when compared to classmates, this value rose to 41%. Disabling cerebral palsy occurred in 12% of the children. Only 20% of the children had no disability including cognitive, neurologic, hearing and vision problems, 34% had mild disablity, 24% moderate and 22% had severe disabilites. Severe disabilities were defined as non-ambulant cerebal palsy, an IQ score 3SD below the mean, blindness and profound hearing loss [33]. The Epipage study of 22–32 week gestation infants born in nine regions in France revealed that at age 5 years 9% of children had cerebral palsy, 32% had a cognitive score < 85 and 12% had a cognitve score < 70. Disability was highest for the children born at the lowest gestational ages (24–28 weeks gestation). Special health care resources were used for 42% of children born at 24–28 weeks gestation and 31% born at 29–32 weeks compared to only 16% born at term [34]. When the 23–25 week gestation children of the Epicure study were compared to those of the Epipage study the rates of cerebral palsy were similar at age 2 years (21% *vs* 16%), as were the rates of cognitive score < 70 at age 5 years (10% *vs* 14%, respectively) [35].

Analyses of outcomes by birth weight rather than gestational age similarly reveal that < 1 kg birth weight children and especially those of 500–750 gram birth weight have significantlly poorer outcomes than normal birth weight controls [36–38]. Impact on the family of having a preterm child which includes financial impact, as well as caretaker and family burden is significantly higher than that of normal birth

weight controls. However it is related mainly to the chronic and neurodevelopmental problems the child may have [39, 40]. Despite the increase in chronic conditions and associated functional disablity which persist among extremely low birth weight adolescents, both parents and the adolescents themselves rate their health related quality of life, in general, similar to that of normal birth weight controls [41]. The findings of a self perceived health related quality of life similar to that of controls persists in young adulthood [42].

18.6 Late Preterm Children

Late preterm children are defined as those born between 34 and 36 weeks gestation. Due to their physiologic and metabolic immaturity they are at higher risk of neonatal morbidities than term born children. These include respiratory problems, temperature instability, hypoglycemia, jaundice and poor feeding. Although information about the later development of the late preterm infant is sparse, these children have been noted to require early intervention services during infancy similar to those of <32 week gestation infants [43]. At school age they have have lower reading and math skills and higher rates of enrollment in special education than term born children. These differences are gestational age dependent with 32–33 week gestation infants having poorer outcomes than those born at 34–36 weeks [44].

18.7 Young Adult Outcomes

The adult outcomes described to date pertain mainly to preterm infants born in the 1970s and early 1980s, a time when neonatal mortality was high and few extremely immature infants survived [45]. The studies suggest that the neurodevelopmental and growth sequelae of prematurity identified during the school age and adolescent years persist into young adulthood. The two major predictors of adult outcomes are lower gestational age, which reflects perinatal injury, and family sociodemographic status, which refects both genetic and environmental effects. When compared to normal birth weight controls, very low birth weight young adults have poorer educational achievement, more chronic illnesses such as asthma or cerebral palsy and less physical activity. Pregnancy and childbirth rates are lower for young adult women. There is also evidence that the young women have higher rates of anxious, depressed and withdrawn symptomatology than controls. However, despite the significant differences, the vast majority of preterm survivors born during the early years of neonatal intensive care do well and live fairly normal lives. Of interest also is the fact that very low birth weight young adults demonstrate less risk-taking than controls including alcohol and drug abuse.

18.8 Early Intervention

Enrichment programs for low birth weight children have included neonatal in-hospital infant and parent focused programs as well as infant educational enrichment and parent support programs during infancy and early childhood [46, 47]. However, long-term beneficial effects have not been demonstrated. A recent meta-analysis of the effectiveness of early developmental intervention programs on cognitive and motor development of preterm children revealed improved cognitive outcomes in infancy and preschool, but no significant impact at school age. There was also no improvement in motor outcomes during infancy or the school age periods [48]. Studies of early physical therapy have similary not been found to have an effect on the rates of cerebral palsy. The longest follow-up to date of early intervention of preterm infants is that of the Infant Health and Development Program in the United States that followed a cohort of low birth weight infants to age 18 years. At this age, although slight improvements in mathematics, behavior and vocabulary were noted for those who participated in the intervention, they were only evident in larger birthweight preterm children with birth weights of 2–2.5 kg. There had been earlier improvements in outcome for the <2 kg birth weight infants suggesting that the long-term effects of early educational enrichment programs may be less effective for infants of lower birth weight and gestation [49, 50].

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19

Early Markers of Poor Outcome

Fabrizio Ferrari, Licia Lugli, Isotta Guidotti and Marisa Pugliese

A major issue for neonatologists and developmental neurologists is the identification of those infants who are at risk of subsequent neurodevelopment disability and who may benefit from neurological follow-up and early intervention strategies.

Currently brain ultrasound (US) is the most widely used imaging technique in neonatal intensive care: serial US have been used in large population-based studies to monitor the rate of cerebral palsy and to investigate the type of brain injury. It is a cheap, useful and relatively easy to use method for the detection of hemorrhagic and cystic lesions, but it is not sensitive enough for diffuse white matter (WM) abnormalities or basal ganglia/thalami (BGT) lesions.

Magnetic resonance imaging (MRI) can offer more detailed information about white matter (WM) and gray matter abnormalities and is the gold standard for neuroimaging techniques during the newborn period. However, it is not easy to perform in the sick neonate and experience in interpretation of the results is limited to a few centers [1]. A large proportion of infants with brain abnormalities seen on MRI do not develop cerebral palsy or major developmental disabilities. Neuroimaging needs to be accompanied by an accurate clinical assessment of the functional repertoire of the infant, which varies according to the stage of development.

A thorough neurological assessment in the neonatal period and during the first two years of life is mandatory in to evaluate neurological deficit and functional impairment due to the brain injury (or a brain defect).

19.1 Poor Outcome

Outcome can be categorized into severe and moderate-to-mild deficits. Severe deficits comprise CP, mental retardation (developmental quotient of less than 70), and severe visual or hearing impairment. Severe deficits can be detected within the first two years of life. Moderate to mild deficits, however, are often not detected until school-age [2].

19.1.1 Cerebral Palsy

Cerebral palsy (CP) is a major disability which is defined as a group of disorders of movement and postural control caused by a nonprogressive defect or lesion of the developing brain. The leading prenatal and perinatal risk factors for CP are birth weight and gestational age, but other risk factors include neonatal encephalopathy, multiple pregnancy, infection and inflammation, and a variety of genetic factors.

CP is in part a developmental diagnosis, and it is based on the description of motor signs that are disabling; such a clinical diagnosis is not easy to make, especially early in development. CP is divided into three major types to describe different movement impairments; this classification also reflects the areas of the brain that are damaged [3]. The three major type are:

- *Spastic CP*: Children with this type of CP are hypertonic and have a neuromuscular condition stemming from damage to the corticospinal tract or the motor cortex. Spastic CP is further classified by topography dependent on the region of the body affected: spastic hemiplegia with one side being affected; spastic diplegia with the lower extremities affected and less upper-body spasticity; spastic tetraplegia with all four limbs affected equally. Monoplegia, paraplegia, triplegia may be used to refer to specific manifestations of the spasticity.
- *Ataxic CP*: Symptoms can be caused by damage to the cerebellum; some individuals have hypotonia and tremors, motor skills might be affected, as well as balance, especially while walking.
- *Athetoid/dyskinetic CP*: This is characterized by mixed tone and sometimes by hypotonia (hypotonia will usually

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

occur before 1 year of age; the muscle tone will be increased with age and progress to hypertonia). People with athetoid CP have trouble holding themselves in an upright, steady position for sitting or walking, and often show involuntary movements. The damage occurs to the extrapyramidal motor system and/or pyramidal tract and to the basal ganglia (BG) [3, 4].

In the 2004 an international meeting on the definition and classification of CP was held in Bethesda. The major addition to the classic Bax and Mutch et al [3] definitions involved emphasis on the accompanying developmental disturbances that accompany neurodevelopment disabilities: these are often functionally more important than the developmental motor disorders that are, by definition, the hallmark of CP [3]. The severity of CP is nowadays classified according to the Gross Motor Function Classification System for Cerebral Palsy (GMFCS): it is a reliable and predictive internationally adopted method of scoring gross motor function in children with CP [4].

19.1.2 Mental Retardation

Mental retardation (MR) or intellectual disability is defined as a disability that involves significant limitations both in intellectual functioning and in adaptive behaviour, which covers many everyday social and practical skills. This disability originates before the age of 18 years and encompasses a wide range of conditions, types, and levels.

19.1.2.1 Visual Disability and Hearing Impairment

Visual disability is the impairment of visual functioning even after treatment and/or standard refractive correction, with a visual acuity in the better eye of less than 6/18 for low vision and 3/60 for blindness or with a visual field of less than 10 degrees from the point of fixation (according to the WHO definition).

A hearing impairment or deafness is a full or partial decrease in the ability to detect or understand sounds. It can be classified as conductive or sensorineural hearing loss [6].

19.2 Traditional Neurological Examination

A variety of standardized examination tools have been developed to estimate gestational age, detect neurological abnormalities and determine their course during the neonatal period and later on during the first 2 years of life [5]. Infants at high risk of brain damage may be included in follow-up programs comprising serial neurological examinations in order to identify early indicators of abnormal neurological development. A simple and scorable neurological examination for infants between 2 and 24 months of age was developed in the Paediatric Department of the Hammersmith Hospital in London (the Infant Neurological Examination [HINE]) [3]. The assessment consists of different items assessing cranial nerve function, posture, movements, tone, reflexes, the development of motor function and the state of behaviour [5]. For details, see Chapter 130.

The prognostic power of the HINE optimality score was explored in very preterm infants between 9 and 18 months of age: 48% had suboptimal scores, the scores were not associated with the degree of prematurity and had a very high accuracy to predict walking at 2 years. The scores were also not invariably associated with the pattern of ultra-sonogram findings: cystic periventricular leukomalacia (PVL) was associated with low scores, other ultra-sonogram abnormalities with both optimal and suboptimal scores. This is not surprising because hemorrhages and periventricular densities can be associated with both normal and abnormal neurodevelopment. A high correlation between the HINE at 3 months and the developmental quotient at 24 months was found. The high predictive value of the HINE across the first year of life was probably granted by the effective combination of different groups of items for each age period. Indeed, although a number of items were found to be consistently among the most predictive ones (movement quality, quantity and lateral tilting), other items changed across time. Tone items (ventral suspension, arm pronation/supination and scarf sign), tended to be more predictive at 3 and 6 months post-term, while maturational items, such as arm protection, vertical suspension and forward parachute, were highly predictive only after 9 months. Therefore these studies demonstrated that a standardized neurological examination performed from 3 to 12 months can be used reliably in very preterm infants to predict motor outcome [6].

The predictive value of the HINE examination was also evaluated in infants with other neurodevelopmental risks, like infants with hypoxic-ischemic encephalopathy (HIE) (see Chapter 136).

Follow-up studies have demonstrated that the outcome cannot be always predicted by the severity of HIE. Although HIE stage 1 is usually associated with a normal outcome and HIE stage 3 with severe neurodevelopmental abnormalities, the outcome in infants with HIE stage 2 can be quite variable, ranging from normal to CP and severe MR. Several studies have reported that neurodevelopmental outcome is best predicted by the pattern of lesions observed on neonatal MRI (see Chapter 132). The HINE was used in a group of infants with HIE at age 9 to 14 months to evaluate the correlation between the scores and early MRI findings, and whether the scores can be used to predict locomotor function at 2 and 4 years. Approximately 40% of the infants had suboptimal scores on neurological examination and the magnitude of the suboptimal scores was related to the pattern of the MRI lesion. Severe BG lesions, diffuse brain injuries, or both were associated with the most severe outcome [7].

The use of the optimality score gave prognostic information on the severity of the functional motor outcome. Although neonatal MRI can identify early infants who will have CP, the neurologic examination can provide information on the severity of the functional motor impairment and distinguish infants who will walk from those who will only sit or not even acquire the sitting posture. It is interesting, however, that few children who were able to walk without restrictions at 4 years had an abnormal gait, associated with a mild hemiplegia or with mild movement disorders, which suggested that mild neurologic abnormalities can also occur in the absence of widespread lesions or severe abnormalities. Further studies evaluating follow-up at school age are required to evaluate whether these minor neurologic abnormalities are related to any difficulty in everyday life [7].

19.3 Early Markers of Cerebral Palsy: the Role of General Movements

The type of brain injuries most frequently occurring in term infants are hypoxic-ischemic injury to the BGT, parasagittal injury; and in preterm infants PVL, intraventricular hemorrhages (IVH) and persistent flare.

CP occurs in 8–10% of very preterm babies and approximately 40% of all children with CP are born preterm. In preterm infants spastic diplegia prevails, followed by hemiplegia. In full-term infants tetraplegia and hemiplegia are by far the most common forms; dyskinetic CP is more common in full-term infants [2].

The early prediction of CP will lead to the child receiving neurological follow-up and earlier enrolment in rehabilitation program. It is generally reported that CP cannot be diagnosed before several months after birth or even before the age of 2 years. A so-called silent period, lasting up to few months has also been described.

The neurological signs observed during the first months after birth in preterm infants who will develop CP are neither sensitive nor specific enough to allow for a reliable prognosis. Irritability, abnormal finger position, spontaneous Babinski reflex, weakness of the lower limbs, transient abnormalities of tone and delay in achieving motor milestones are some of the neurological signs that have been described in these high risk preterm infants. All of them may be encountered before the onset of CP or during transient dystonia, dissociated motor development, and other transient neurological disturbances that vanish during the first or second year of life.

In the last two decades a technique for assessing spontaneous motor activity has been introduced and evaluated, namely Prechtl's assessment of general movements (GMs). This approach, which involves observation of the infant without direct physical examination, has proved a reliable and sensitive method in the neonatal period and early infancy for predicting normal and abnormal motor outcome, particularly CP [8]. It requires video-recording of a few minutes of spontaneous motility and off-line observation of the quality of GMs. Serial assessments of GMs from preterm birth until 3–5 months post-term age define the developmental trajectory. Assessment based on developmental trajectory predicts CP at a much earlier age than other neurological assessment.

19.3.1 Diplegia and Tetraplegia

Early specific markers for spastic cerebral palsy are cramped-synchronized (CS) GMs and abnormal fidgety GMs. As far as CS GMs, the movements of limbs and trunk appear rigid and lack normal smooth and fluent character. Limb and trunk muscle contract and relax almost simultaneously. All infants who show consistent and prolonged CS GMs later develop CP. The earlier consistent CS GMs occur, the worse the later motor impairment of preterm infants. If the CS character is transient and followed by normal fidgety GMs the infant may have a normal outcome [9]. The second specific marker for CP is absent fidgety movements (FMs). Almost all infants who never had FMs developed CP. Absence of FMs can be preceded either by CS GMs or by poor repertoire (PR) GMs. Both abnormal qualities of GMs, i.e., CS GMs and/or absence of FMs, are seen within 3–5 months post-term, thus much earlier than with other neurological assessment [9].

19.3.2 Hemiplegia

Hemiplegia (HE) is usually due to focal one sided lesions, either ischemic or hemorrhagic infarction, more rarely to cystic lesions due to focal or unilateral PVL.. Brain US and MRI detect site and severity of the focal lesions: the neurological follow-up of these infants aims to detect (or exclude) early signs of HE. Traditional neonatal neurological examination is of low predictive value for such patients.

The studies carried on with the GMs method have shown that all babies with a focal lesion have an abnormal quality of GMs (PR or CS) from term, and, a silent window is not observed. Asymmetrical segmental movements can be recognized from as early as 3 months post-term in preterm infants and from 3–6 weeks post-term in full-term infants with neonatal cerebral infarction. Segmental movements are distinct wrist movements, occurring either in isolation or as a part of GMs.

In term infants who later develop HE asymmetry in wrist movements, a lower frequency of independent digit movements are seen as early as 12 weeks and predict later HE. It is important to note that only infants with concomitant ipsilateral involvement of a cerebral hemisphere, BG and the internal capsule on the MRI brain scan later develop HE. Early recognition of subtle motor asymmetries is not easy with unaided eyes; it is easier when videorecording of spontaneous motor behavior is available [10].

19.3.3 Dyskinetic CP

Dyskinetic CP has a frequency of only 10–15% of all CP forms. The dyskinetic form of CP comprises two subtypes: a syndrome of choreatetosis (infants showing mainly massive, purposeless involuntary movements) and a dystonic form, characterized by sudden shifts of muscle contraction and infantile reflex activity. This second subtype is more common. During the first two months, infants who will develop a dyskinetic form, display a PR of GMs, arm movements in circles and finger spreading. Abnormal arm and finger movements remain until at least 5 months and are associated with lack of arm and leg movements towards the midline (particularly foot-foot contact) and with lack of FMs. The abnormal unilateral or bilateral circular arm movements are monotonous, slow forward rotations from the shoulder. The monotony in speed and amplitude is the most characteristic quality of circular arm movements.

An early differential diagnosis of dyskinetic versus spastic later CP is not easy: later dyskinetic infants share the absence of FMs and antigravity movements, i.e., leg lifting in infants with later spastic form. Moreover, circular arm movements can be mistaken for wind-milling arm movements seen during the first year of life and with cycling movements described in infants with HIE. Choreo-athetoid movements are easy to recognize but the sudden shifts of muscle contraction are difficult to differentiate from the abnormal muscle tone of spastic forms. Dystonic and spastic features are quite commonly mixed in the same infant [11].

19.4 Neuroimaging Predictors

Besides neurologic assessment of the infant with neonatal brain injury or at risk for neurodevelopmental disturbances, neuroimaging can provide predictors of neurodevelopmental outcome. Two main imaging techniques have been used for the prediction of neurodevelopmental outcome in the neonatal period: US and MRI (see Chapter 132).

19.4.1 Cerebral Ultrasound

US is a simple bedside tool and when used repeatedly, its sensitivity and specificity can be very high, expecially in the preterm infant. US lesions can be detected in the majority of infants born below 32 weeks of gestation who develop CP. IVH grade III and IV, PVL and focal infarctions are major cerebral lesions detected by US in the preterm population. Also infants with a grade I-II IVH are at an almost two times higher risk of developing neurodevelopmental impairments at 20 months compared with those without any hemorrhage. The degree of milder US findings (grade I or II PVL) and size of the corpus callosum are also related to neuromotor function in school-age children without CP. New techniques such as transmastoidal access have improved the representation of the cerebellum, a structure that can also be injured in very low birth weight (VLBW) infants. Cerebellar injuries may contribute to long-term neurocognitive disabilities, independent of associated supratentorial parenchymal lesions [2].

Cerebral US is useful in the critically ill newborn who cannot be studied with MRI. During the first hours after asphyxia, US may give little information on the severity of lesions and prediction of outcome. Arterial Doppler studies (measurement of the pulsatility index) may better predict outcome, but in most cases it is still normal 6–12 hours after the insult. The most frequent lesion observed by US in asphyxia is BGT hyper-echogenicity while parasagittal ischemia is more difficult to detect, because the distance between brain lesions and the acoustic window is high.

19.4.2 MRI Abnormalities and Outcome

MRI of the preterm infant is best done at term equivalent age when the myelination of the posterior limb of the internal capsule may be used as a predictor of motor outcome. MRI lesions in the full-term infant are best seen after the first week.

In the preterm infant, MRI has been used to detect diffuse WM abnormalities and gray matter changes. Gray matter abnormalities are also associated with the same risks but to a lesser extent than WM abnormalities. WM abnormalities can be detected in more than 50% of VLBW children and are predictive of CP and psychomotor delay in early childhood and neurosensory impairment. Recently it has been shown that HIE, typically described in in full-term infants, can also be observed in pretem infants. These infants have a high incidence of severe BGT and brainstem involvement and the lesions are associated with significant mortality and neurologic morbidity. Advanced MRI techniques such as volumetry and morphometry, diffusion tensor imaging and tractography have been used to better determine the full spectrum of brain injury. In infants born preterm and examined at term, abnormalities in the cortex and deep nuclear structures are related to the degree of immaturity at birth and concomitant WM injury. Volumetric changes in the sensorimotor, premotor, midtemporal and parieto-occipital regions may be related to intellectual performance at school-age. Recent findings indicate that the hippocampal volume may be reduced in preterm infants and that it is associated with reduced working memory, cognitive and motor performance at 2 years of age. However, the significance of subtle abnormalities detected on MRI regarding long-term functioning has not yet been fully resolved [2].

HIE and BGT are often associated with abnormal signal intensity in the posterior limb of the internal capsule (PLIC), brainstem, hippocampal region and cortex, particularly around

the central fissure and insula. Loss of the normal signal intensity from the PLIC is associated with adjacent BGT injury. The motor correlate is a spastic or dyskinetic CP. BGT lesions are the imaging signature of an acute and profound hypoxic ischemic event. BGT lesions associated with severe WM damage, conversely, suggest a more prolonged hypoxic-ischemic insult. WM damage is proved by a loss of gray matter/WM differentiation, consistent with an overt infarction: in these infants a very poor outcome can be expected.

When the predominant lesion involves the WM and cortex but spares the BG and PLIC, the pattern probably results from prolonged partial hypoxia-ischemia: the motor outcome is often good but there may be cognitive and behavioral impairments that are proportionate to the extent of WM and cortical injury. Severe BGT lesions are associated with a high risk of developing CP and severe cognitive impairment regardless of the additional associated WM lesions [12].

19.4.3 Cognitive Evaluation

The most widely used standardized developmental scales to assess mental development, in the first two years after birth are the Bayley Scales of Infant Development (BSID) and the Griffiths Mental Developmental Scales.

The new Bayley Scales of Infant and Toddler Development, third edition, include updated items and modernized stimulus materials and manipulatives, and yield mental assessment results as separate cognitive and language scores rather than as separate scores for Cognitive, Language and Motor scales and separate scores for Expressive and Receptive Communication and for Fine and Gross Motor skills. The associated Adaptive-Behavior parent questionnaire is an excellent addition to the new Bayley scales, giving functional information that can be used to support a tested significant mental delay. On the other hand, the Griffiths Mental Development Scales for children from birth to 24 months, reviewed in 1996, comprises items to assess locomotor, personal-social, hearing and language, eye and hand coordination, and performance domain. Domain raw scores are obtained and converted to standardized sub-quotients, percentiles and developmental age, the sum of which is the General Quotient, a score for an overall developmental level.

The purpose of developmental standardized scales is to assess a child's development at the time of observation, to determine developmental delay or impairment, in order to implement early habilitation interventions. Despite the wide use of standardized scales to determine the extent of developmental delay or impairment, major concern remains about their accuracy in discriminating cognitive function. To evaluate specific cognitive deficits, it is essential to use neuropsychological tests and to continue cognitive follow-up at least until school age. Developmental scales are not intelligence tests and developmental quotients (DQ) calculated in the first two years are not as reliable as the intelligence quotient (IQ) measurement, calculated after the first 3 years of life, nor do they have as good a predictive value for later IQ. However, in preterm populations, a score of 2 standard deviations (SD) below the normative mean has been used as a cut-off to identify abnormalities. Currently, following a consensus statement, many authors suggest that a cut-off of 3 SD is more appropriate and may be more predictive of later poor performance [2].

19.5 Visual Evaluation

Subcortical visual pathways have a major role in neonatal vision while cortical visual modules progressively emerge during the first months of postnatal life. Abnormal visual function occurs in infants with brain lesions even in the absence of ocular abnormalities (cortical blindness). Blindness affects early motor development, especially self-initiated postures and locomotion.

Term infants with HIE can show visual abnormalities (reduction in visual acuity, fixation shift or visual fields in the first years of life. Occipital cortex and optic radiation injury have been found in infants with visual impairement, but cortical and WM lesions sparing the BG are not always associated with abnormal visual findings. Cortical and BG lesions are always associated with severe abnormalities of visual function during the first year of life, confirming the role of BG in human visual development. Poor acuity, weak stereopsis and delayed visual maturation can occur in chidren with focal BG lesion [13].

VLBW infants are at risk of visual, visuocognitive and visuomotor impairment. Vision loss for preterm infants is given as visual impairment with the best vision after correction of < 20/60, or legal blindness with the best corrected vision of < 20/200 (< 6/60). Severe visual impairment occurs in 1–3% of ELBW and 2–6% in children with gestational ages below 27 weeks.

Retinopathy of prematurity (ROP), is one of the main causes of poor visual function and blindness; prevention is based on ophthalmic screening to identify cases requiring treatment. Less severe visual impairments include refractive errors, reduced visual acuity, strabismus and nystagmus. IVH and PVL are other causes of visual impairment [14]. Severity of visual impairment is also related to the extent of basal ganglia, lateral geniculate nuclei and optic radiation involvement. Fractional anisotropy of the optic radiation was related to visual assessment scores, independent of WM maturation.

Assessment of visual function at term corrected age includes: ability to fix and follow a target, preferential looking, attention at distance, color and stripe discrimination. Measures of early visual cortical function are orientation reversal visual event-related potentials (OR-VERP) and fixation shifts under competition. Both measures correlate with the severity

of brain abnormalities and are sensitive predictors of neurodevelopmental outcome at 2 years.

Post-discharge eye examinations until preschool are useful to monitor refractive errors and to prevent amblyopia.

19.6 Hearing Evaluation

Hearing loss (HL) is one of the most common birth defects (3/1000) and is associated with language and perception disorders. Genetic factors, prematurity, low birth weight, hyperbilirubinemia, pharmacologic ototoxicity and noise exposure are the main causes of HL. The introduction of universal screening resulted in a significant decrease of HL.

Otoacoustic emissions (OAE) and auditory brainstem response (ABR) are employed in newborn hearing screening programs. The OAE measures the inner ear response to sound by placing a microphone in the ear canal. The ABR records the brain's response to sound by placing electrodes on the head.

Auditory steady-state responses have been reported as a reliable and objective technique for evaluating the hearing threshold. ABR and auditory steady-state responses in infants with perinatal brain injury show a high sensitivity of auditory steady-state responses for detecting hearing impairment.

In term infants much hearing impairment is due to specific gene abnormalities, including the connexin mutation. Some authors state that hypoxia-ischemia damages cochlear hair cells, causing hearing impairment. Infants carrying genetic mutations for hearing loss may coincidentally experience perinatal stress, but prevalence of hearing impairment in children born with HIE is not significantly increased.

In preterm infants, sensorineural HL has been associated with prolonged ventilation, ligation of patent ductus, hypotension and ototoxicity, infections, craniofacial abnormalities and family history. All extremely low birth weight (ELBW) infants are considered at high risk for HL, and in most centers hearing testing is done prior to intensive care discharge, either as OAE or full diagnostic testing with ABR. Occasionally, this test defines auditory neuropathy with abnormal auditory brainstem function and a strong cochlear microphonic with normal OAE.

Although as many as 6% of children with gestational age below 26–27 weeks have severe to profound hearing impairment, many function well after the early placement of cochlear implants.

Children with undiagnosed HL can experience delays in the development of language, social, and academic skills. Besides the degree of hearing loss and age at onset of deafness, environmental factors (parental support and education methods) seem to play an important role in the cognitive development and academic success. As language development begins within the first months of life, it is important for parents to be aware of normal language and communication developmental milestones beginning when their baby is very young so that they can discuss development with their child's healthcare provider [15].

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20

Quality of Neonatal Intensive Care and Outcome for High Risk Newborn Infants

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It is generally accepted that outcome is related at least in part to the quality of care and therefore the quality of care delivered to babies should be closely monitored in order to improve outcome. Quality in neonatal care is a complex subject, but improving and developing the quality of care is a goal to which all neonatologists should aspire. In order to achieve incremental improvement in quality of care, these outcomes need to be monitored ongoing interim measures need to be implemented in order to improve care such as audit, benchmarking and confidential enquiries, which are discussed below. Traditional measures of outcome such as death, neurodevelopmental status and chronic lung disease are also discussed in detail later in this chapter. Improving quality of care can additionally be achieved by implementing modern, effective, evidence based methods of knowledge translation in order to increase the use of evidenced based health care.

20.1 What Is Quality in Neonatal Care?

The quality of the care that we provide to all newborn babies has gained increasing prominence in the last decade or so. Quality in healthcare has been defined in many different ways and by many different authors. However, appertaining to neonatal care it has recently been defined as:

> [...] providing an appropriate level of care to well newborn babies while providing a good quality of more specialised care to the few babies that need it. This should be done while obtaining the best medical neonatal outcome and satisfying parents and families as well as providers. This should be delivered in the context of sound managerial and financial performance and whilst developing perinatal services to further raise standards of care. [1]

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This definition clearly demonstrates the complexity of measuring quality in neonatal care; not only do we need to consider the care that the baby has received and the effects of that care, but also the effect on the family, parents and health care professionals surrounding that baby. Compounding these factors with the complexities of healthcare management and economics makes measurement of this highly complicated entity very difficult.

20.1.1 Measuring Quality in Neonatal Health Care

The assessment of quality in neonatal care is evolving and the framework around which quality can be measured is that of clinical governance. This encompasses many different processes by which care can be measured.

In the past, outcome studies have been used for reflecting the quality of care, but more recently other methods have become more commonplace such as auditing, benchmarking and confidential enquiries. Subsequently, increasing amounts of work are being published on ways in which to improve quality of care, based on the published literature.

When measuring quality it is important to recognize and differentiate the discrete aspects of healthcare – the structure or organization of care that is provided, the process of providing that care and the outcome of the care that has been provided [2]. These different aspects may be assessed individually to measure quality.

20.1.2 Outcome Studies

In the past much of the neonatal literature has been focused on different aspects of care and their effect on outcome for a baby. Outcome can mean many different things, such as later neurodevelopmental attainment, and the presence or absence

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

of chronic lung disease. These outcome measures can be used as a proxy for the quality of care.

One of the problems with using studies reporting outcome as a marker for quality of care is that there are frequently differences in results between similarly designed studies. These differences can be in part accounted for by differences in clinical practice or in the demographics of the study populations. However, it has been shown that these differences in clinical practice and in demographics are not enough to explain the variation in results. It has been suggested that the differences may therefore be due to a difference in outcome in quality of care [3].

20.1.3 Audit

Clinical audit is a quality improvement process that seeks to improve the patient care and outcomes through the systematic review of care against explicit standards and subsequently the implementation of change. Where indicated, changes are implemented and further monitoring is used to confirm improvement in healthcare delivery. It is a continuous circular process. Therefore, audit does not define what optimal care is, but whether perceived optimal care is actually provided.

In the UK the National Neonatal Audit Programme (NNAP) was set up in 2006 and uses the audit method to capture information on current practice measured against previously defined standards. The first annual report highlighted many of the problems encountered when auditing such a complex process as healthcare [4]. Three years data has now been reported. In light of the results of the first period, changes were made to the questionnaire and also to data ascertainment. However, there continue to be issues with interpreting the data and ensuring that the questions asked give the information required.

The report highlights examples of difficulties in the analysis of data as a result of imprecise questions. One standard set by consensus of the NNAP Board was "of those receiving surfactant, 90% should receive it within 1 hour of birth". In answer to the audit question of whether this standard was achieved, there was data missing from 173/647 babies. Of all eligible babies 64% received surfactant in the first hour (87% of babies with complete data) showing that the standard was not achieved. However, the question could not distinguish those babies who did not receive surfactant in the first hour because they were not intubated, following the increasingly common occurrence of babies of this gestation going directly onto nasal continuous positive airways pressure (nCPAP) after birth. Similar issues were highlighted with the remaining questions. However, despite the problems encountered the quality of early surfactant administration is clearly not up to the expected standard and so changes can be made to improve this.

Within Europe, the Euronatal Study [5] investigated differences in perinatal mortality rate by looking at quality of care in a geographical region within 10 individual countries. Differences were demonstrated between different health care systems. Suboptimal care factors occurred significantly less in Finnish and Swedish regions than in the remaining participating regions (Spain, the Netherlands, Scotland, Belgium, Denmark, Norway, Greece and England). Sixty seven per cent of neonatal deaths ≥ 34 weeks were graded by an expert panel to have had suboptimal care factors that were likely or might have contributed to the fatal outcome. Clearly, this study showed that improvements in quality of care should be possible but no re-audit has been carried out to date.

When auditing a standard of care if the expected standard has not been met change should be implemented to achieve that standard. Re-audit is then imperative in order to demonstrate that change has been beneficial and the quality of care has improved.

20.1.4 Benchmarking

Benchmarking is a process whereby aspects of health care practice are evaluated against known best practice. It is a helpful way to compare activity and performance in different areas with other organizations and to provide an evidence base for further review and action planning. Benchmarking is a commonly used technique across a wide range of different sectors and industries to review and improve performance. It is one of the most widely applied management techniques used to improve organizational performance.

A useful definition of benchmarking is provided by The NHS Benchmarking Club [6], who define the process as "Making evidence-based comparisons of practice, drawing conclusions and implementing improvements".

Benchmarking has many benefits. It is an evidence based quality improvement tool. It can provide the means to compare practice with similar organizations and thereby identify outliers from the standard of care, i.e., good and bad performers. It can assess whether current performance achieves practice standards and can provide the catalyst for organizational change to improve quality of care. Sharing benchmarking data can share good practice and motivate the need for change. The first large benchmarking collaboration in neonatal medicine was within the Vermont-Oxford Network (VON) and now provides benchmarking data for participating units internationally. The UK Managed Clinical Networks (see below) can provide a group of similar units that can benchmark both internally and externally.

20.1.5 Confidential Enquiries

Standard methods of measurement (e.g., audit) may not be sufficiently powerful to capture the complexity of neonatal

health care. A more focused approach may sometimes be needed such as that used in Project 27/28 [7], which was carried out in England, Wales and Northern Ireland in the 2 year period between September 1998 and August 2000. The aim was to identify variations in the standards of care that might have contributed to the deaths of infants born at 27 and 28 weeks gestation during this period. Anonymized medical records were scrutinised by a panel of experts for deficiencies in the standards of care provided to the infants that died. Having highlighted consistent deficiencies in the standards of care the working party published recommendations for changes to practice in order to improve the quality of care. Their main findings and recommendations were based upon early thermal, ventilatory and cardiovascular care.

20.2 How Can Quality be Improved in Neonatal Care?

20.2.1 National and International Groups

Different groups whose aim is to improve quality within neonatal care have been set up. The largest organization is the Vermont Oxford Network (VON). This is an international collaborative of neonatal units with the objective of integrating research and clinical practice in order to improve the quality of neonatal care [8]. Other organizations such as the Neonatal Survey (UK) and Australian and New Zealand Neonatal Network (ANZNN) collect data and use this to provide information on outcome. Contibutors to these databases are then able use the information provided for developing and enhancing their quality of care.

20.2.2 Managed Clinical Networks

Managed Clinical Networks are a way of delivering high quality care to a population across a large geographical area. A Managed Clinical Network has been defined as:

> Linked groups of health professionals from primary, secondary and tertiary care, working in a co-ordinated manner, unconstrained by existing professional and Trust/Health Authority boundaries, to ensure equitable provision of high quality and clinically effective services. [9]

Managed Clinical Networks in the UK were set up after a government initiative called "The NHS Plan". The aim was to create a more effective and efficient service. The network should ensure appropriate access for patients to the right care delivered in the most relevant setting. Access to specialist knowledge and care should be streamlined and quality of care should be consistent [10].

The network can play a central role in the maintenance of national standards for neonatal care but can also develop and implement local quality improvement measures such as taking a leading role in the development of education, audit and guidelines [11]. The networks can also provide support and advice for individual units about both clinical and non-clinical matters. All these network roles clearly can improve quality.

20.2.3 Education and Knowledge Translation

It is well described that there are major gaps between routine practice and what the evidence shows is optimal practice [12]. An example of this is nicely demonstrated by the work done by Ligand in the early 1970s that showed the improvement in respiratory function of newborn premature lambs and later human preterm infants after antenatal maternal steroid administration. Although the benefit was clearly demonstrated it took until the late 1990s to reach routine antenatal care.

Translating audit and research findings into best practice is hampered by many barriers and obstacles at all levels of care delivery. This has been addressed in several studies that show that using active methods of information dissemination (e.g., audit, feedback, multidimensional workshops, ongoing support) knowledge translation can be far more effective than traditional methods of learning such as lectures and seminars [13, 14].

20.3 Short-Term and Long-Term Morbidity and Mortality in High Risk Infants

Infants born prematurely are at significant risk of both increased morbidity and mortality compared with babies born at term [15]. They represent one of the leading causes of perinatal morbidity and mortality in the developed world [15–17].

Reported rates of preterm delivery (<37 weeks) in North America are 12–13% [17], compared with 5–9% in Europe [18]. The highest morbidity and mortality not surprisingly is seen in the smallest, most premature infants [16, 19–23]. These figures are considerably higher in the developing world [24].

Premature delivery therefore continues to be a significant problem worldwide and rates show no evidence of declining; in contrast there are many reports worldwide of increasing rates of preterm birth [15, 16, 25–27]. A variety of factors have contributed to the increasing preterm birth rate, including increased use of assisted reproduction techniques and the associated increase in multiple births [15, 16, 18]. Increases have also been seen in the rates of singleton preterm birth particularly amongst medically induced preterm births rather than those born following the spontaneous onset of labour [15, 18]. In addition singletons born following assisted

reproduction are more likely to be preterm than their spontaneously conceived counterparts [28]. Socio-economic status is well described to have a significant effect on preterm birth; however this effect has been shown to be static over time [26]. Racial origin also impacts on rates of preterm birth; changes in racial demographics over time may therefore have influenced rates of preterm birth [22, 29, 30]. Reported rates of preterm birth may have also have been influenced by changing attitudes in delivery units over time with more infants being classed as live births or offered resuscitation [31, 32], however this may not be the case at all gestational ages [33].

20.3.1 Mortality

Major advances in antenatal, delivery suite and neonatal management of preterm infants led to major advances in survival up until the mid to late 1990s when compared with the 1980s or earlier [20, 25, 34–36]. Contributors to improved survival include the increasing and now widespread use of antenatal corticosteroids [20, 23, 25, 36, 37], changes in attitude in delivery units to the management of extremely preterm infants and widespread postnatal use of surfactant [36, 38]. There is now a wide range of international data from the mid to late 1990s following widespread introduction of antenatal corticosteroids and postnatal surfactant reporting mortality for the preterm infant for geographically based studies.

More recently in the late 1990s and into the 21st century the changes in survival have been less convincing [20, 36, 39]. Recent large studies in the UK show some improvement in survival in extremely preterm infants born after 24 weeks but not at lower gestational ages [33, 40]. However in other European countries the trends towards increased survival have continued into the 21st century [41].

Table 20.1 shows the reported survival for extremely preterm infants from three international cohorts.

Preterm birth at 34–36 weeks of gestational age accounts for 75% of all preterm births [42, 43] and whilst such infants have a lower morbidity and mortality than those born extremely prematurely, they still have a significant mortality and morbidity when compared with those infants born at term [42]. Mortality rates were significantly higher at 34 (1.1/1000 live births), 35 (1.5/1000) and 36 (0.5/1000) weeks gestation when compared with the term infants (0.2/1000).

Although the number of infants born at < 32 weeks represent < 5% of all babies born, most mortality is seen in this group, particularly the 0.4% of all infants born at less than 28 weeks. Infants born at 25 weeks are 32 times more likely to die than if born at 31 weeks [44]. Within this highest risk group separate data is available for the smallest most premature infants. Multicenter data from the UK for births in 2006 shows 25% mortality for infants born at 22–23 completed weeks (of those admitted) [41]. Higher rates of survival of 40% of live births for those born at 23 weeks have been shown in a multicenter Swedish study [45]. Data from a single centre in the USA gives a survival (as a percentage of all births) of 1.8% at 22 weeks increasing to 34% at 23 weeks [19].

20.3.1.1 Multiple Births

As a result of the well described increase in multiple birth rate over the last 20 years, concerns have been raised regarding the potential for increased mortality and morbidity in such infants compared with their singleton counterparts. In a large, nationwide Canadian study of infants < 32 weeks, there was no increase in mortality in those infants born following multiple births when compared with gestationally matched singletons [46]. These findings are supported by other large studies [47–49]. In addition, different measures of morbidity were comparable apart from a higher rate of respiratory distress syndrome (RDS) in those infants born following multiple birth [46]. This effect was seen only in the second twin in one large study [48].

20.3.1.2 Gender Effects

Male infants born extremely preterm are consistently shown to have a higher mortality than their female counterparts [20, 40], with some authors reporting females showing generally a week's additional maturity and a birth weight heavier than 100 g than their male counterparts [50]. This negative effect of male sex is pervasive into early childhood with higher rates of neurological and developmental disability when compared with females of the same age [51].

Table 20.1 Survival at limits of viability from three large cohorts

	Survival 22+0–22+6 wks	Survival 23+0–23+6 wks	Survival at 24+0–24+6 wks
Sweden [45]	Not available	(at 180 days) 40% of live born infants	(at 180 days) 50% of live born infants
United Kingdom [38]	(to discharge) 9% of admitted infants	(to discharge) 20% of admitted infants	(to discharge) 33% of admitted infants
United States [19]	(to discharge) 4.5% of live born infants	(to discharge) 46% of live born infants	(to discharge) 59% of live born infants

Table 20.2 Major variations in mortality data for different developed countries

Country	Type	Date	Number	Gestation	Survival
Belgium [47]	National	1999–2000	322 (liveborn)	22–26	54% liveborn to discharge
Denmark [62]	National	1994–1995	407 (actively treated)	22–27+6 +/– <1 kg	56% actively treated
Norway [23]	National	1999–2000	636 (all births)	22–27+6 or 500–999 g	59% of all births
Finland [56]	National	1996–1997	529 (all births)	22 weeks, <1000 g	55% of all birth including still births
France [54]	National	1997	4395 (all births)	22–32 weeks	85% of all births
UK & Ireland [38]	National	1995	4004 (all births)	20–25+6	39% of admissions
USA [72]	Regional	1995–1996	4438 (all births)	501–1500 g	84% of all births by d28
Australia [34]	Regional	1991–1992	401 (live births)	23–27 weeks	56% of live births to 2 years

20.3.1.3 Geographical Variations

There are notable inter-country and inter-regional variations in overall mortality of extremely preterm infants. Frequent comparisons are made between different regions/countries, but such comparisons can be misleading. Crude perinatal mortality rate used alone will be significantly influenced by the rate of preterm birth [52] which varies considerably between regions within Europe [53]. Despite using mortality specific for preterm infants there will remain major variations which may not be a reflection of true mortality [44]. Table 20.2 highlights major variations in simple crude mortality data. Denominators vary for all infants including those terminated, to those admitted to the neonatal unit for ongoing care following delivery suite resuscitation. Such variations were highlighted by the French EPIPAGE study which showed an overall survival rate of 67% for all births < 32 weeks, but 85% for live births and 89% admitted to intensive care [54]. Mortality will also be influenced by the attitude and approach of staff at the time of delivery [31, 32]. Such proactive delivery suite policies, whilst resulting in low mortality, have not necessarily been associated with increasing burden of short or long-term morbidity [32].

20.3.2 Morbidity

As with mortality the smallest most immature infants are at highest risk of adverse outcome [21, 55, 56]. With the increased rate of survival seen in the 1990s following extremely preterm birth, it was anticipated that a significant increase in survivors with morbidity would be seen. Many authors have shown morbidity in survivors to be static over time rather than increasing, but with the increase in total numbers of survivors the absolute numbers of those with morbidity will undoubtedly have increased [20, 25, 35].

When interpreting the wide range of studies examining morbidity in the preterm infant, one must be cautious with the interpretation of results, as the denominator used clearly will affect the results: the proportion of infants with a particular clinical condition may be a percentage of the total number of infants born, the total number admitted for intensive care and for longer term morbidities may be a percentage of the total survivors.

Morbidity in moderately preterm infants (30–36 weeks) is less than those born most preterm, but as these deliveries occur in much higher numbers than extremely preterm deliveries, absolute numbers are much higher and therefore such infants do impact significantly on neonatal intensive care units. As infants then become more mature towards term, morbidities gradually decrease [42, 57, 58].

20.3.2.1 Lung Disease

Respiratory distress syndrome is a frequent early marker of respiratory morbidity in preterm infants. This may then evolve into chronic respiratory symptoms termed bronchopulmonary dysplasia (BPD) or chronic lung disease (CLD). Definitions of BPD/CLD generally define this as either oxygen dependence at 36 weeks' post menstrual age (PMA) or at 28 days after birth. More recently definitions of BPD have been refined in order to provide more useful information and have been subclassified into mild, moderate and severe BPD [59].

As with mortality and other morbidities the highest rates of respiratory disease are seen in the smallest most immature infants with over 90% of infants < 500g having RDS [55]. As birth weight increases from 500 g to1500 g, reducing rates of RDS are well described [20]. In infants born prior to 28 weeks' gestation rates of RDS range from 80–90% [38, 47, 54].

Whilst such morbidities are seen less frequently in the less premature group (30–36 weeks), they are still seen more frequently in this group as compared to term infants. Rates of respiratory distress in infants born at 34 weeks are 5–6% with a step-wise reduction at each gestational week to a risk of below 1% at term [42]. Within the less mature group aged 30–34 weeks, respiratory distress syndrome again reduces at each gestational age week with rates of > 40% at 30 weeks as compared to < 3% at 34 weeks [57].

The now widespread use of antenatal steroids (ANS) has significantly reduced the risk of the need for respiratory support and respiratory distress syndrome in the premature infant [60]. Postnatal surfactant therapy also improves survival and reduces risk of pneumothorax [36]. Despite this, RDS rates over time are frequently reported as static despite significant

increase in the use of both ANS and surfactant. For example, rates of RDS in 1991 through to 1999 in a large multicenter USA study in infants 501–1500 g were static [36]. In other temporal comparisons of population cohorts, RDS rates have been shown to increase in more recent years [39]. RDS has a wide clinical phenotype and it may be that cases over recent years following the widespread use of ANS and surfactant may be less severe, with fewer infants requiring invasive respiratory support and less associated mortality [36], but this may then result in more BPD [25].

Rates of BPD/CLD across Europe vary somewhat, but in infants born at < 32 weeks the rates range from 10–20% in survivors to discharge [53]. Rates of CLD as high as 74% are reported in the smallest infants with birth weights of 401–500 g [55]. Between 23 and 25 weeks, rates of CLD significantly decrease from 65% at 23 weeks to around 30% at 24 and 25 weeks [19]. If birth weight is used, rates decrease significantly from nearly 50% in those infants 501–750 g to 6% in those infants of birthweight 1251–1500 g. Overall, in this population of infants weighing 501–1500 g, BPD develops in 22% [20].

Of infants born at less than 26 completed weeks in the 2006 UK EPICure cohort, 75% were oxygen dependent at 36 weeks corrected age [40]. Disappointingly, this figure is not significantly different to the previous EPICure cohort from the 1990s [38]. A comparison of two cohorts from the late 1990s and early 2000s also showed little difference in the rates of BPD with rates of just over 50% (< 1 kg, < 28 weeks), despite increased ANS and postnatal surfactant use over the 2 time periods [61]. Some authors have shown rises in rates of BPD despite significant increases in the use of ANS and surfactant [25]. Over recent years the timing of surfactant administration has been optimized, with early prophylactic use in the extremely preterm infant and this optimal practice may be reflected in improvements in respiratory morbidity over coming years. Lack of improvement may also be explained by a different causative pathology as compared with the pre-surfactant era and as such current infants with BPD/CLD have a different pathogenesis and hence disease than those in previous eras. Pathological findings of the "new" BPD have shown fewer and larger alveoli, indicating a possible defect of septation as compared with previous findings of inflammation and fibrosis seen pre surfactant [59].

The choice of ventilatory support may influence rates of subsequent chronic lung disease. A large multicenter study of infants <1 kg and <28 weeks in Denmark showed rates of chronic lung disease of 16% in those infants managed with CPAP and permissive hypercapnia [62]. In the recently published multicenter trial of CPAP *vs* intubation in infants <29 weeks there was no difference in BPD or death in the two groups [63]. BPD may then act as a predictor of other poor outcomes. At 2.5 years those infants with CLD have an odds ratio of > 2 for cerebral palsy [51]. These findings are supported by other studies showing an odds ratio of 2.5 for death or neurosensory impairment at 18 months if BPD is present at 26 weeks corrected gestational age [64].

20.3.2.2 Brain Pathology

Early neurodevelopmental morbidity can arguably be monitored by rates of intraventricular hemorrhage (IVH) and/or cystic periventricular leukomalacia (PVL) detected on cranial ultrasound scan (CUSS). The incidence of CUSS abnormalities vary significantly between countries and within regions of Europe [53]. Some variation will be seen as a result of user variability differences in the denominator; higher rates being seen in non-surviving infants [65]. In those infants with more severe IVH, intra-observer variability has been shown to be less when compared with minor abnormalities [66]. This intra-observer variability in the more minor abnormalities may explain some of the increases in less severe IVH described recently by some authors [25]. Modern equipment with higher resolution may also influence detection rates of minor IVH detection.

Following improved perinatal care in recent decades and specifically the widespread introduction of antenatal steroids, a reduction in the rates of IVH has been reported [36, 65, 67]. Other reports show static rates of IVH over time [35, 40, 56, 61] or possibly a trend towards a higher incidence of less serious IVH [25]. However, whilst antenatal corticosteroids may reduce the risk of IVH, subsequent neurodevelopmental outcome may not be affected [37]. Other authors describe a neuroprotective effect of ANS, with a protective effect against more severe motor disability at 2.5 years [51].

Within Europe the rate of severe IVH/cystic PVL in preterm infants (< 32 weeks) varies widely from 2.6% to 10% [53]. Comparisons of individual data sets can be difficult as definitions of abnormality and inclusion criteria vary. Data from 2008 in the UK shows a 15% rate of abnormal CUSS in survivors born at 26 weeks or less, which is unchanged from 1995 [38, 40]. Multicenter French data for infants born < 32 weeks reported a rate of 3% for IVH with dilatation, rising to 20% for minor IVH without dilatation and 21% for any white matter damage including both those with the most severe cystic PVL and those with minor persisting echodensities [68]. Comparable multicenter data from Belgium showed that 11% of infants < 27 weeks had major IVH, 25% had a minor IVH and 10% cystic PVL [47]. Multicenter data from Denmark showed a comparable figure for severe IVH/PVL in survivors < 28 weeks [62].

In keeping with all morbidities associated with extreme prematurity, rates of IVH/PVL increase with decreasing gestational age. Those at the limits of viability with the lowest birth weights are most affected, with rates of IVH/PVL as high as 84% at 23 weeks [19]. Other series report rates of white matter damage (WMD) of 37% at 23/24 weeks decreasing to 17% at 30 weeks. Minor IVH shows a similar trend with a rate of 40% at 23/24 weeks decreasing to 22% at 30 weeks. Between 30 and 34 weeks there is a statistically significant decreasing trend for IVH/PVL. In those more mature preterm infants, IVH is < 1% (all grades) in 34–36 week infants, but still significantly greater than the rate seen in term infants [41].

The effect of early abnormalities seen on cranial ultrasound scan on long-term neurodevelopmental outcome is variable. Abnormal last CUSS in the large EPICure 1 cohort gave an odds ratio of 4.95 for cerebral palsy. However this was not a sensitive marker of long-term disability with less than 50% of infants with cerebral palsy at 2.5 years having white matter changes on cranial ultrasound scan on the neonatal unit [51]. However a significantly abnormal final cranial ultrasound scan independently increased the risk of cerebral palsy [51]. Follow-up of the large multicenter French cohort showed that 25% of those with white matter damage on CUSS had cerebral palsy at 2 years versus 4% of those with a normal CUSS. One third of children with cerebral palsy had a normal CUSS [69].

20.3.3 Longer-Term Outcome

Neurological and developmental morbidity data from discharge up into later childhood and adulthood is now available from some of the large multicenter studies. The large UK EPICure cohort (22–25 completed weeks) showed rates of severe disability at 2.5 years of 23% with an additional 25% having some disability including abnormalities of sensory and communication function, mental and psychomotor development. Nineteen percent of these infants born at 25 completed weeks or less had cerebral palsy at 2.5 years [51]. Lower rates of cerebral palsy of 8% are seen at 2 years in a large French cohort, however, this cohort included infants up to a gestational age of 32 weeks [69].

At 5 years, 9% of children born very preterm in the large cohort reported from France had cerebral palsy, 39% had some disability with 5% being classified as severe, 9% moderate and 25% minor. Not surprisingly the risk of disability was highest in the 24–28 week group but absolute numbers were greater in the more mature infants up to 32 weeks [70]. In keeping with these findings, the risk of disability at 5 years in the less premature infants (30–34 weeks) reduces with a rate of 6% for cerebral palsy in infants born at 30 weeks and further decreasing significantly to < 1% in babies born at 34 weeks [57].

Significant long-term neurodevelopmental effects of prematurity have been described well into adulthood. A large population based study of babies born at less than 27 weeks had a relative risk of 10.3 for mental retardation when compared to their contemporaries born at term. The same group had a relative risk of 7.5 of receiving a disability pension when compared with those born at term [71]. In addition, the gestational age of adults in this large population study was associated with their educational attainment, income and receipt of social security benefits. Those born preterm achieved a lower educational level, income and were significantly more likely to be in receipt of social security benefits. These findings highlight the pervasive effects of prematurity well in adulthood.

20.3.3.1 Visual Disorders

Retinopathy of prematurity (ROP) is frequently used as a marker for morbidity in the high risk preterm infant. It can be challenging to report comparable figures as studies report different outcomes including all ROP, severe ROP only or treatment for ROP. As infants become more mature rates of ROP reduce gradually with infants of higher gestations being very unlikely to need treatment for ROP [40, 47, 56]. In infants born at less than 29 weeks almost one third had some ROP with 14% requiring treatment for this 23. Other studies of infants <27 weeks reported 20% requiring treatment for ROP [47]. In the smallest most preterm infants (401–500 g) rates of ROP reach as high as 89% with one thirds of all infants in this cohort having severe ROP. Treatment for ROP in the 2 Epicure cohorts (1995 versus 2006) showed a significant increase from 13 to 21%, but it is likely that this increase represents a lower threshold for treatment rather than a true increase in morbidity [38, 40]. When ROP is combined with two other morbidities, BPD and brain injury, not surprisingly the three together were strongly predictive of death or neurosensory impairment at 18 months [64].

20.3.3.2 Other Outcome Measures

Significant additional morbidity is seen in preterm infants influencing both their short- and long-term clinical course and outcome. Necrotising enterocolitis (NEC) continues to be a significant risk to the extremely preterm infant and a source of significant mortality and short- and long-term morbidity. Some temporal comparisons have shown a slight but insignificant decrease in rates of NEC over time [35, 56], but generally rates remain static at between 5 and 10% for extremely preterm infants [20, 25, 36]. The highest rates tend to be seen in the smallest infants [72] and decrease with increasing maturity to rates of less than 0.1% at 34 weeks, but such rates remain significantly higher than those seen in term infants [42].

Sepsis in the preterm also continues to be a significant source of morbidity and mortality. Early onset sepsis is seen in up to 15% of infants born extremely preterm [20, 47] with a mortality of 37% in these infants as compared with 13% in those infants in the cohort without early onset sepsis [20]. Some authors have suggested an increase in sepsis in such infants over time [35], but late onset sepsis appears to have remained static over time with rates of around 20% for infants 501–1500 g [20]. The smallest preterm infants (401–500 g) had very high rates of sepsis, with over half having at least one episode and late sepsis reported in one quarter [55]. Again, as with other morbidities those less premature infants whilst at less risk of sepsis than their extremely preterm counterparts are at greater risk than their term equivalents, with rates at 34 weeks of 0.5% versus 0.1% at term [42]. Episodes of sepsis can then be subsequently associated with other morbidities, for example early sepsis has been shown to increase the risk of IVH [67] and chronic lung disease [62].

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21

Cerebral Plasticity and Functional Reorganization in Children with Congenital Brain Lesions

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21.1 Introduction

Cerebral plasticity plays an important role during brain development. For example, children learn a new language or achieve complex skills, such as playing a musical instrument, faster than adults. In a classic experiment on string players, the extent of the cortical representation of the left digits was found to be inversely correlated with the age at which the person had begun to play, indicating a larger amount of cerebral plasticity in subjects with earlier exposure to training [1]. Similarly, children lacking proper environmental inputs early in life are more likely to have abnormal development of the functions related to those inputs (the concept of critical periods) [2].

The presence of mechanisms for neuronal plasticity during early phases of development should mean that recovery from brain damage is better for early lesions compared to similar lesions acquired later in life. This principle was first suggested by Paul Broca in 1865 [3] and then systematically explored by Margaret Kennard in the late 1930s [4]. Since then, studies carried out in different species have not refuted this general principle. The picture appears, however, to be more complex. Account needs to be taken of factors other than the timing of the insult; these include the location and extent of injury (e.g., focal or diffuse), the clinical correlates (e.g., presence of seizures), and the genetic susceptibility of the subject [5].

One of the most important predictors of the efficacy of functional reorganization seems to be the distribution of the damage, i.e., whether diffuse or focal [6, 7]. Strikingly, children with early unilateral left hemisphere damage may develop normal language, while lesions at a similar site and of similar extent in the adult brain result in aphasia [8]. Even if an entire hemisphere is removed at an early stage of development (for instance for the treatment of severe epilepsy), children can develop normal language and cognitive function [9]. Also, children with unilateral ischemic stroke are able to develop normal cognitive functions, which they maintain over time [10].

By contrast, children who sustain an early generalized cerebral insult (e.g., global hypoxia or traumatic brain injury) recover slower with a poorer outcome, compared to adults with similar lesions [11]. The mechanism most often invoked for this greater vulnerability to early damage is that, at an early stage, cognitive development is highly dependent on the integrity of diffuse neural networks; thus the transient disruption of developing attention, memory, and learning functions undermines the effective acquisition of new abilities [7]. Conversely, at an older age, cognitive abilities, which have already been acquired, may be spared and impairment is limited to functions directly related to the final area of damage.

The aim of this chapter is to outline recent discoveries relating to mechanisms of functional reorganization in the young brain, in view of future possible non-pharmacological therapeutic interventions.

21.2 How Early is Early?

The time boundaries of early brain damage have never been clearly defined. This is due to the complexity of the task rather than to lack of effort. Cerebral plasticity, which influences the effects of brain damage, has a gradual effect during development and vulnerable periods are now known to be different for the various functional sub-systems [2]. The types of brain insults vary during development and affect the nervous system in ways that depend on the level of maturity at the time they occur. The boundaries between early and late lesions are therefore necessarily fuzzy. This chapter explores the differences of the young brain's response to damage and focuses on lesions occurring before or around birth (also called congenital lesions), which are more frequent and have been more extensively studied than later ones.

A relevant aspect of the pathophysiology of congenital brain injury is the stage of cerebral development at the time of

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

the insult, which may be either prenatal or perinatal. Because of the complexity and speed of maturation during pregnancy, the response to a harmful event varies with gestational age with different neuropathological and clinical outcomes. Our understanding has been enhanced by non-invasive neuroimaging techniques, first ultrasonography, and, more recently, computerised tomography (CT) and magnetic resonance imaging (MRI). These new methods, increasingly applied to children, allow *in vivo* investigation of cerebral lesions by monitoring their evolution and providing further insight about the relationship between lesion and its effect on function.

In broad terms, types of congenital brain damage can be grouped according to timing (Fig. 21.1). Lesions occurring during the first half of gestation (and in particular the first trimester) give rise to cortical malformations. These lesions can vary in size, location and distribution, resulting in different clinical pictures. The underlying mechanisms of cerebral plasticity are likely to be similarly variable. The second group of lesions are those occurring around the early third trimester of gestation (approx. 25–34 weeks of gestation). The most typical of this group are periventricular leukomalacia and intra-ventricular hemorrhage. The first is a diffuse lesion due to ischemia or infection-inflammation, while the second is a hemorrhagic lesion, usually limited to within the ventricles, but sometimes developing into a periventricular parenchymal infarction, usually unilateral. The third group includes lesions around term (typically lesions affecting the term infant at around birth). The most relevant are hypoxic-ischemic encephalopathy (HIE) and focal cerebral stroke. HIE is a bilateral and diffuse ischemic lesion, while a stroke is a focal lesion of arterial origin, with similar neuropathology to the stroke observed in adults.

The distribution of the lesion, i.e., focal unilateral compared with diffuse bilateral, appears to be the single most important factor that influences the effectiveness of the plastic reorganization of brain function. Congenital lesions therefore provide an interesting model for studying cerebral reorganization because they include different combinations of distribution (focal, diffuse) and timing (early gestation, late gestation and term).

21.3 Cerebral Plasticity and the Different Systems

The effects of a cerebral lesion are related to the site of the lesion. This implies different involvement of the various systems in different subjects, with complex and heterogeneous functional correlates. Although subjects with cerebral damage that is acquired early show a clinical phenotype consisting of impairment of language, sensori-motor and visual systems, we will consider each system independently, focusing on cerebral plasticity and differentiating the effects of early and late lesions.

21.3.1 Language

Language processing generally (i.e., in 95% and 98% of adults) occurs in the left hemisphere of the brain. How this special ability develops, its nature and what happens when the left hemisphere is damaged by a cerebral injury during early development are still matters of debate. More than 30 years ago, invasive techniques such as the Wada test demonstrated that language developed in the right hemisphere of patients with early left hemispheric brain lesions [12]. How this happens and the consequences for brain function are starting to be clarified by the application of advanced functional neuroimaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI).

An important aspect relates to where (and indirectly how) in the right hemisphere language function is organized. In 2002, Martin Staudt and coworkers used fMRI and a language task to explore the topography of right-hemispheric language organization after early left brain injury by unilateral periventricular lesions. They showed a remarkable similarity between activation in the left hemisphere in normal controls and activation in the right hemisphere in brain injured patients, with identical distribution of the known cortical areas of the language circuit [13]. These findings indicated that reorganization of language in the right hemisphere occurs in areas that are homotopic (i.e., in the same location) to those

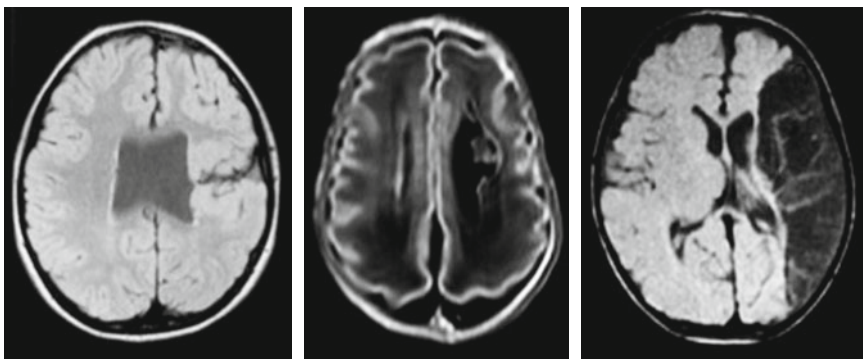

Fig. 21.1 Correlation between timing of insult and type of brain injury. Three examples are shown of typical congenital brain lesions on MRI; the correlation between timing and characteristics of the lesion are clear. In the *left column*, a case of schizencephalia, a malformation of cortical development secondary to an insult acquired during the early phases of brain development. In the *central column*, a case of periventricular white matter injury, secondary to an intraventricular hemorrhage, occurred at the beginning of the third trimester of gestation. In the right column, a case of ischemic infarction in the territory of the middle cerebral artery, occurred around birth in an infant born at term

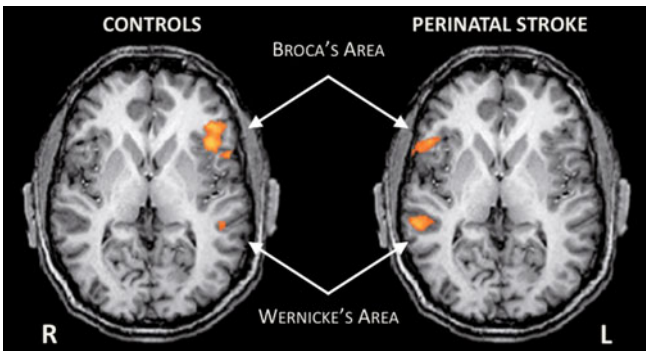

Fig. 21.2 Language representation in patients with left perinatal stroke and right hemispheric reorganization of language. fMRI shows the activation of regions in the right hemisphere which are contralateral and homotopic to the regions of the language area, activated in normal controls (a group analysis was performed on 8 patients and 10 controls). Data from [16]

normally involved, strongly suggesting a near-equipotentiality of the two hemispheres at birth as far as the ability to develop language control is concerned.

Similar findings were found in patients with malformations of cortical development [14, 15]. In those subjects, however, epileptic seizures, which are known to alter cerebral reorganization, were almost invariably present. This made it hard to explore whether shifting language processing could also happen in the absence of seizures and/or with later damage (early third trimester). More recently, Guzzetta et al demonstrated a similar pattern of contralateral homotopic reorganization in patients with arterial stroke at term (Fig. 21.2) [16]. The study also showed that a shift of language function after a stroke at term is more common than for earlier lesions, suggesting a direct influence of timing on the pattern of reorganization. If this pattern is confirmed, it might suggest that, as for other systems (see below), the hemispheric specialization of language develops because of competition between the two hemispheres. If a left (non-epileptogenic) lesion occurs during the early third trimester when cerebral plasticity is highly active, the affected hemisphere is more likely to maintain its genetic advantage over the contralateral hemisphere and eventually develop control over language. When a left lesion occurs at term or during early development when the potential for plasticity is reduced, the non-affected hemisphere might take over language development. This possibility becomes progressively less available during later development, with later lesions invariably resulting in an intra-hemispheric reorganization of function and different degrees of language disturbance.

21.3.2 The Sensori-Motor (SM) System

When a cerebral cortical or subcortical lesion involves the motor system, neuroplastic mechanisms should be able to drive recovery of voluntary movements, restoring an adequate cortical impulse to the spinal motor neurons and inter-neurons. In the case of a cerebral lesion, two major mechanisms are available for restoring an efficient re-connection of the motor cortex with the spinal cord. The first involves reorganisation of the ipsilateral cortex within the primary motor cortex or in non-primary motor areas. The second mechanism is specific for lesions during early development. It is based on the existence of bilateral motor projections originating in the primary motor areas during the first weeks of life post-term; these connect each hemisphere with both sides of the body. These fibres withdraw during later development, but may persist in the case of cerebral damage, giving rise to a contralesional reorganization of motor function, but this mechanism is limited to early brain damage (Fig. 21.3).

fMRI is able to provide relevant information on the type of reorganization occurring in each patient. Integration with other techniques to provide temporal resolution, such as Transcranial Magnetic Stimulation (TMS), demonstrates the existence of cortical-spinal monosynaptic connections. TMS has shown that in subjects with early lesions of the motor cortex, there is significant bilateral corticospinal innervation of spinal motoneuron pools and that these persist in the healthy hemisphere. In these subjects, activation of the intact motor cortex elicits large responses in both ipsi- and contra- lateral muscles, with similar latencies and thresholds.

But what are the consequences of having found this specific type of motor reorganization after early damage? It appears that this pattern of SM reorganization (contralesional reorganization) is determined during the first year of life, and possibly even within the first few months [17]. This is not only a consequence of the size and site of the lesion, but is strongly influenced by what happens after damage (action

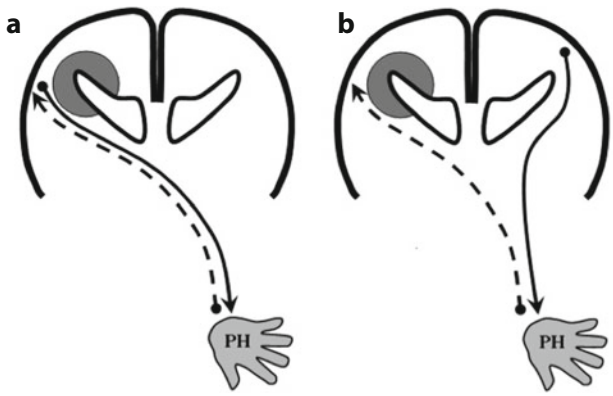

Fig. 21.3 Main types of reorganization of sensori-motor function following early brain damage. **a** Ipsi-lesional reorganization of motor and sensory function. Both functions are reorganized in the affected hemisphere, in regions around the lesion. In this case, functional impairment was mainly related to the extent of damage to the sensori-motor system. **b** Contra-lesional reorganization of motor function and ipsi-lesional reorganization of sensory function. Motor and sensory functions of the affected limb are processed by different hemispheres. In this case, functional impairment is related not only to the extent to which the sensori-motor system has been damaged, but also to the presence of a functional dissociation

dependent reorganization): there is a complex interaction between the residual motor output from the affected hemisphere and a somato-sensory feedback from the affected limb – the hypothesis has been called "amblyopia of the corticospinal system" [17]. Confirmation of this hypothesis would emphasise the importance of an early time window (the first months of life) for therapeutic intervention. This is especially true when considering that children with contra-lesional reorganization, i.e., when the unaffected hemisphere directly controlling both hands, achieve less good hand-motor performance, making this pattern of reorganization potentially maladaptive [18, 19].

Cerebral lesions affecting the motor system often involve the sensory system as well, and may lead to a functional deficit. These functions can be studied *in vivo* with techniques like somato-sensory evoked potentials, magnetoencephalography and fMRI with sensory stimulation. These approaches have demonstrated that, by contrast with the motor system, the intra-hemispheric (ipsilesional) reorganization of primary sensory function is the principal, if not the only, compensatory mechanism for brain damage of the sensory system, even when this occurs very early during development [19].

The mechanisms underlying this phenomenon are not fully understood. However, two elements seem to be of special relevance. The first is the lack of an anatomical substrate for contralesional reorganization, even during the early stages of development, in contrast to what happens to the motor system. The second is the possibility that at least for some types of early lesions, thalamo-cortical fibres are still developing when the insult occurs, thus allowing a bypass of the lesion and reconnection with the sensory cortex [20].

It is of considerable interest that the different reorganizational potentials of the sensory and the motor systems often result in an inter-hemispheric dissociation of these functions, with the sensory system being reorganised in the affected hemisphere and the motor system being shifted contralaterally (Fig. 21.3b). There is some evidence to support the hypothesis that such a dissociation could lead to functional deficits in tasks requiring good sensory-motor integration (such as stereognosia). In light of these findings, a specific target of early therapeutic intervention might be activation of the sensori-motor cortex of the affected hemisphere to enhance the competitive ability of a damaged corticospinal system during development and, by so doing, to mitigate the consequences of injury on motor function.

21.3.3 The Visual System

There have been few investigations of reorganization of the visual system after early lesions in humans. However, visual function has been more studied in animals, especially the cat, than any other system. The sparse scientific evidence from humans on the specificity of reorganizational mechanisms after early damage is summarized below (Fig. 21.4).

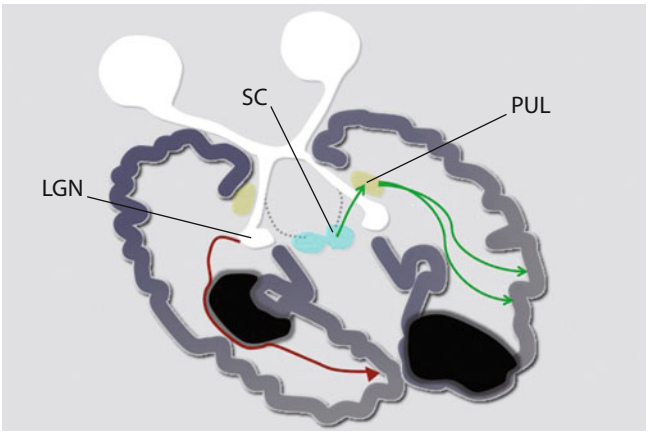

Fig. 21.4 Main types of reorganization of visual function following early brain damage. On the left, a possible type of reorganization following early damage of the optic radiations. The thalamo-cortical connections are able to bypass the lesion and reach the final cortical target (primary visual cortex). A full recovery of conscious vision can be achieved. On the right, the reorganization mechanisms following early damage to the primary visual cortex. Circuits connecting the retina with extrastriatal visual structures are expanded, in particular involving the superior colliculus and the pulvinar. A full recovery of conscious vison cannot be achieved, but a high degree of functional compensation is obtained, consisting of near normal exploratory visual behaviour and navigation. *LGN* Lateral Geniculate Nucleus, *SC* Superior Colliculus, *PUL* Pulvinar

The correlation between damage to the optic radiations or the occipital cortex and the corresponding visual field deficit is far less strong in the case of an early lesion than for a lesion occurring later in life. This might be a direct expression of greater cerebral plasticity in the young child. It may, at least in part, have a similar neurophysiological basis to that observed for the somato-sensory system; in particular there is a possibility that thalamo-cortical fibres develop after the injury and so bypass it. The precise characteristics and limits for plasticity involving thalamo-cortical connections are not fully understood. Some data suggest that up to term, structural modifications of the geniculo-striate pathway enable functional reorganization of the visual system. A combination of fMRI and diffusion tensor tractography was used in a recent longitudinal study of an infant with a perinatal left arterial stroke [21, 22]. The stroke spared the primary visual cortex but involved the optic radiations. At 3 months, cortical activation could only be observed in the unaffected side; diffusion tensor imaging (DTI) was unable to show the presence of optical radiations in the affected hemisphere. At 20 months, the infant was re-tested using the same protocol and surprisingly showed definite fMRI activation, an indirect sign of functional reorganization. This was further supported by clear structural modifications on diffusion tractography [22]. Unfortunately, assessment of visual fields could not be performed because of the subject's young age. However, regardless of the possible presence of functional impairment, the imaging data seem to support the existence of a process of reorganization at the level of the thalamocortical pathway, and an ability to restore at least

partially a functional connection between the lateral geniculate body and the occipital cortex.

Even when there is a visual field deficit, patients with early damage seem to have fewer difficulties in environmental navigation and exploration. These data are in line with findings from animal models, which showed clearly that ablation of the whole primary visual cortex in the newborn animal did not affect visual orientation, which, by contrast, was massively impaired after a similar lesion in adult animals. Studies on cats showed that this phenomenon is linked to reorganization of the pathways connecting subcortical visual structures (the lateral geniculate nucleus, superior colliculus and pulvinar) directly to the extrastriatal ipsi- and contra- lateral visual centers [23]. This could also apply to humans in some certain degree, as shown, for example, by increased activation on fMRI of extrastriatal structures after stimulation of the affected hemifield. Reorganization of visual function appears to be more effective after early brain damage. This may be due either to reconnection with targeted structures or to the use of compensating circuits. Even if such circuits are not able to restore conscious visual perception on the contralesional hemifield, they seem to allow for good compensation in spatial orientation and localisation. These conclusions are being confirmed by a study in progress at the Stella Maris Scientific Institute; this is aimed at demonstrating how, in a visual search task, congenital hemianopic subjects perform normally, whereas patients with acquired hemianopia are significantly compromised.

21.4 Conclusions

How can we summarize present knowledge on cerebral plasticity and reorganization following early brain damage? The emerging concept, based on studies on both human and non-human subjects, is that functional reorganization in children is similar to that in adults, but there are differences. An understanding of the specific mechanisms of cerebral plasticity in infancy is far from complete. Such knowledge will, however, be essential for the definition and development of therapies based on sound neurobiological and neurophysiological principles. Information is needed about not only the type of treatment, but also its timing, dosage, and means of administration. Brain mapping techniques, and in particular fMRI, have provided answers to many questions. Implementations of other technologies, for example Ultra High Field MRI tomography, will doubtless provide further insights.

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22

Organization of Perinatal Care

Neil Marlow

22.1 Overview

Medical and nursing care for the newborn is organized differently in different health systems. Whereas general support in terms of screening and routine care is provided for a large number of babies in a maternity setting, neonatal intensive care for sick and immature babies is a low throughput high cost service. The development of neonatal intensive care has undoubtedly been one of the success stories of modern medicine. Its rapid evolution since the early 1970s has led to dramatic changes in mortality at low gestational ages and a reduction in immediate and later morbidities. Nonetheless, babies born at "borderline viability" (gestations with >50% mortality) have consistently demonstrated a very high morbidity rate, even though the "borderline" gestational age is reduced and currently sits at around 23–25 weeks in most developed countries.

The extent to which different models of organization of neonatal services have contributed to this is hotly debated. Services evolve as the technical aspects of care change and are rarely planned on a geographic basis. However, there is a general agreement that a sick neonate needs to be treated in a specialist neonatal unit. Thus, a neonatal transport system must be a basic element for any regional program that aims at reducing morbidity and mortality (see Chapter 24).

Organization is very dependent on how the healthcare system is financed and managed and on the regulatory framework of a particular country. In this chapter we describe the recent changes in organization within the United Kingdom healthcare system to illustrate the thinking and evidence base on which service changes may be planned and from which the reader may derive concepts relevant to their own local health system.

Briefly, in the UK, healthcare is organized independently in each of the four constituent countries. Within the UK, the majority of births occur in hospital (approximately 600,000 out of 680,000 annually) and healthcare is organized under the auspices of the National Health Service. The NHS is regionalized into 12 Strategic Health Authorities and they are responsible for "purchasing" health services for their population from Hospital and Primary Care Trusts (the "providers"). Low volume services such as neonatal intensive care are "commissioned" (purchased or contracted) centrally.

Neonatal care is delivered in maternity hospital settings within the UK, except for highly specialist areas, for example such as cardiac surgery. Over recent years, hospitals have become increasingly focused on the areas of neonatal care they undertake based around a three tier model, where some units provide only basic non-invasive care, some more invasive care (usually including short periods of intensive care) and other hospitals provide a full neonatal intensive care service; more specialist services – surgery, extracorporeal membrane oxygenation, cardiac surgery – are provided in only a few centers around England to optimize efficiency and maximize quality outcomes.

22.2 Models of Care

Neonatal care cannot take place in isolation from other services. In particular pediatric, obstetric and fetal medicine services are interdependent so it makes sense that these are co-located. In many non-UK systems, however, neonatal intensive care takes place within a children's hospital setting, which has implications for family integrity and support but may provide optimal support for the highly technical aspects of care and management. In contrast there are increasing moves in some countries to enhance the concept of family-centered care by co-locating maternal and neonatal care for mother and child in the same area. This model does allow for some separation for the periods of necessary intensive care but optimizes the family experience and is designed to minimize psychological morbidity. Neonatal care should be

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

organized in a manner appropriate to the societal setting and resources available.

The issue of centralized versus dispersed services has been the subject of much debate. Apart from research carried out in the UK [1], studies in other health systems have seen advantages in terms of mortality and morbidity where services are centralized to provide care for the sickest and smallest infants in large "tertiary" units [2]. It has been suggested that the reason that this has not been shown in England relates to under-capacity within the large neonatal services [3, 4], as there is evidence that mortality is related to the number of nurses available each shift [5].

To address this problem we have developed a series of "managed clinical networks" of neonatal care. A managed clinical network may be defined as linked groups of health professionals and organizations from primary, secondary and tertiary care working in a coordinated manner to ensure equitable provision of high-quality clinically effective services, unconstrained by existing professional and Health Board boundaries. Further description of the roles and responsibilities of Managed Neonatal Networks is available [2], but in essence all hospitals serving a population agree to work together to deliver the whole range of care. Where possible this is carried out as close to the mother's area of residence but where specialist care (i.e., intensive care) is required then the mother and baby are transferred to the appropriate centre. This way hospitals that provide different levels of care act together to make sure that the baby and family are appropriately supported. To date these networks of care are variously effective but recent developments have been undertaken to formalize their function [6].

22.3 Care of High-Risk Newborn Infants

Within a neonatal service a range of areas require planning and monitoring:

- antenatal counseling and support
- delivery room care and resuscitation
- screening and acute care following "normal" births
- care in the neonatal unit
- discharge care and support
- follow-up of at-risk children.

22.3.1 Antenatal Counseling and Support

Central to good perinatal care is the availability of neonatal staff to discuss forthcoming deliveries with women and their partners. This is a key role for the neonatologist and the neonatal nurse. Discussions may simply include information about the process of birth for an at-risk child and a pre-delivery visit to the neonatal unit. In other situations the neonatologist must be involved in discussions of prognosis where delivery is anticipated very prematurely or where fetal anomaly has been detected and on occasions must be part of the decision-making process as to where a baby is best delivered to receive optimal care. Most fetal medicine services now involve the relevant pediatric specialist, where a fetal problem has been identified to help develop a delivery plan, but of equal importance is the neonatal team who will often have a different perspective on the early management of the baby, which may alter the initial proposal.

22.3.2 Delivery Room Care and Resuscitation

Neonatal staff are usually responsible for the organization and training of all obstetric, midwifery and neonatal staff in neonatal stabilization and resuscitation. In most countries formalized training is available through nationally accredited neonatal life support courses and all staff that are likely to be involved in neonatal stabilization should be properly accredited [7, 8]. Clear written arrangements for checking that resuscitation apparatus is always available and for calling appropriate staff when necessary should be agreed. Management plans where comfort or palliative care is anticipated need to be agreed and, given the ethical difficulties that surround, in particular, birth at extremely low gestations each neonatal unit should agree with their obstetric and midwifery colleagues a policy for communicating risk to prospective parents facing such deliveries.

22.3.3 Screening and Acute Care Following "Normal" Births

Within our health system the neonatologist is responsible for carrying out a screening examination for the newborn baby and troubleshooting where the midwife has identified potential postnatal problems. This involves identifying babies who are at high risk of particular conditions such as neonatal infection, hip dysplasia, and congenital heart disease. Screening for metabolic disease is also undertaken in most countries.

In most hospitals the screening examination is delegated to trainees or to specifically trained midwifery staff, although in some settings it may be undertaken by the family pediatrician. A single neonatal examination is usually sufficient to identify major anomalies, although problems, which become apparent later in the neonatal period such as duct or pressure dependent congenital heart disease and jaundice, may need separate assessments [9]. Screening with saturation monitors for heart disease may enhance the detection rate of screening examinations [10]. Hospitals need to ensure that they have a range of protocols for treating babies at risk of infection [11] and babies who develop new problems after birth [12].

22.3.4 Care in the Neonatal Unit

22.3.4.1 Designation of Neonatal Care and Services

Neonatal units have a range of service available to them that differ from hospital to hospital. Often the quanta of care that is provided depend upon staffing levels and co-located facilities. In many countries a three level system operates formally or informally with a fourth level designating those hospitals which provide "super-specialist" care such as cardiac surgery, neonatal surgery etc. The detail will vary from one health system to another. The British Association of Perinatal Medicine (BAPM) grading [13] is shown in Table 22.1.

Within these designations it is important that we define what activity occurs within each service. Again these definitions will vary widely between health systems. The BAPM definitions of normal, special, high dependency and intensive care are shown in Table 22.2. One of the key features about the use of definitions such as these is that they dictate the level of support required by the baby and that this is independent of the setting in which the care is delivered. For example if a baby required intensive care whilst awaiting transfer to a neonatal unit, the referring hospital should be able to provide the equipment and manpower immediately available to cope with the baby's needs. This may cause problems in that if the staff are needed for an emergency elsewhere in the hospital; other staff members should be available to deal with that leaving experienced staff to care for the baby who needs intensive care. Similarly, often babies who fall into the category "special care" are cared for alongside their mothers. This requires enhanced nursing support but may occur outside the confines of a neonatal unit. Sometimes this is known as "transitional care". Neonatal units have used transitional care as a means of enhancing the care they give (promoting mother-baby contact) and also of enhancing the capacity of their neonatal unit, but need extra resources to do it properly. In our system the nursing requirements also follow the care category as indicated in Table 22.2. Because staff costs are the greater proportion of total unit costs this allows re-imbursement to be made for care on a bed day basis.

Table 22.1 Designation of neonatal services within a neonatal network

Level 1 or *Special Care Units* – provide special care but do not aim to provide any continuing high dependency or intensive care; this includes units with or without resident medical staff
Level 2 or *Local Neonatal Units* – provide high dependency care and some short-term intensive care as agreed within the network; medical staffing at middle grade and consultant level is shared with a general pediatric service
Level 3 or *Neonatal Intensive Care Units* – provide the whole range of medical neonatal care but not necessarily all specialist services such as neonatal surgery; medical staffing at all grades is dedicated to the neonatal service

Adapted from [13].

Table 22.2 Definitions of categories of neonatal care

Intensive care

These babies have the most complex problems. They need 1:1 care by a nurse with a neonatal qualification. The possibility of acute deterioration is such that there should be the constant availability of a competent doctor.

1. Receiving any respiratory support via a tracheal tube and in the first 24 hours after its withdrawal
2. Receiving NCPAP for any part of the day and less than five days old
3. Below 1000 g current weight and receiving NCPAP for any part of the day and for 24 hours after withdrawal
4. Less than 29 weeks gestational age and less than 48 hours old
5. Requiring major emergency surgery, for the pre-operative period and post-operatively for 24 hours
6. Requiring complex clinical procedures:
   - Full exchange transfusion
   - Peritoneal dialysis
   - Infusion of an inotrope, pulmonary vasodilator or prostaglandin and for 24 hours afterwards
7. Any other very unstable baby considered by the nurse-in-charge to need 1:1 nursing: *for audit, a register should be kept of the clinical details of babies recorded in this category*
8. A baby on the day of death

High dependency care

A nurse should not be responsible for the care of more than two babies in this category

1. Receiving NCPAP for any part of the day and not fulfilling any of the criteria for intensive care
2. Below 1000 g current weight and not fulfilling any of the criteria for intensive care
3. Receiving parenteral nutrition
4. Having convulsions
5. Receiving oxygen therapy and below 1500 g current weight
6. Requiring treatment for neonatal abstinence syndrome
7. Requiring specified procedures that do not fulfill any criteria for intensive care:
   - Care of an intra-arterial catheter or chest drain
   - Partial exchange transfusion
   - Tracheostomy care until supervised by a parent
8. Requiring frequent stimulation for severe apnea

Special care

A nurse should not be responsible for the care of more than four babies receiving Special or Normal Care

- Special care is provided for all other babies who could not reasonably be expected to be looked after at home by their mother

Routine care

- Is provided for babies who themselves have no medical indication to be in hospital

Adapted from [13].

22.3.4.2 Family-Centered Neonatal Care

Traditionally parents were observers whilst medical and nursing experts assumed responsibility for care. Indeed many units were designed with viewing galleries or corridors to prevent contact. With the advent of more enlightened views of the role

Table 22.3 Examples of family-friendly standards for NICU

1. Where admission to a neonatal unit is predicted, a pre-natal opportunity to visit the neonatal unit and meet key personnel should be offered to the family
2. All parents should be introduced to facilities, routines, staff and equipment on admission to a neonatal unit
3. Every parent should have unrestricted access to his or her baby, unless individual restrictions can be justified in the baby's best interest
4. Parents should be encouraged and supported to participate in decision-making about the care and treatment of their baby. Written and regularly updated care plans should be shared with parents. Clinical care decisions, including end of life decisions, should be made by experienced staff in partnership with the parents and discussions held in an appropriate setting
5. Parents are encouraged and supported to participate in their baby's care at the earliest opportunity, including:
   - Regular skin to skin care
   - Providing comforting touch, comfort holding, particularly during painful procedures
   - Feeding
   - Day-to-day care, such as nappy changing
6. Every baby should be treated with dignity and respect:
   - Appropriate positioning is promoted and encouraged
   - Clinical interventions are managed to minimize stress, avoid pain and conserve energy
   - Noise and light levels are managed to minimize stress
   - Appropriate clothing is used at all times, taking into account parents' choice
   - Privacy is respected and promoted as appropriate to the baby's condition
7. Every parent will have the opportunity to discuss their baby's diagnoses and care with a senior clinician within 24 hours following admission or a significant change in condition.
8. Written information should be available (in languages and formats appropriate to the local community) to all users of the service on medical and surgical treatments, to permit early and effective communication with parents covering at least:
   - Condition/diagnosis
   - Treatment options available
   - Likely outcomes/benefits of treatment
   - Possible complications/risks
   - Possible tests and investigations
   - Who to contact with queries or for advice
   - Where to go for further information, including useful websites
   - Circumstances requiring consent (written and verbal)
9. Maternity and neonatal services should encourage breastfeeding and the expression of milk through the provision of information and dedicated support, including:
   - whenever possible, initiation of breast feeding as soon as possible after birth
   - when necessary, support to start expression as soon after delivery as the mother's condition allows to maximize the benefit of colostrum
   - the availability of a comfortable, dedicated and discrete area
   - the facility to express discretely at the cotside
   - the availability of breast pumps and associated equipment for every mother who requires them
   - supporting breastfeeding as part of the discharge process
   - promotion of safe and hygienic handling and storage of breast milk
   - possible availability of donor breast milk

Adapted from [11] and [14].

of the parent in enhancing the baby's progress through the neonatal period, parents are now welcomed into the neonatal unit and encouraged to participate in their child's care. The advantages of this in engendering better psychological support for parents are clear and there is evidence that skin-to-skin contact is of great importance in promoting infant stability and well-being. Encouraging close parental involvement in their child's care demands organizational changes in the way neonatal services are run, the facilities available for parents such as sitting rooms and overnight bedrooms, and for privacy when they are with their child [6]. Some units have developed the concept of single cubicle care to accommodate this more appropriately but staffing, space and cost constraints make this an expensive if desirable option.

Within a neonatal service it is possible to draw up a range of standards for family-friendly care to ensure that a baby and his family are afforded due respect and that communication and involvement occur. An example of one such set of standards is included in Table 22.3. Importantly these should be auditable so that the neonatal service should be able to demonstrate its commitment to this important area.

Further organizational issues centre around the discharge of the baby home at the completion of the episode of perinatal care. The process of taking a baby home is highly stressful for parents. Units need to develop expertise and clear arrangements for preparing families for home and if possible supporting them in the transition. Information packs, training in resuscitation techniques and supportive outpatient follow-up are important steps in the discharge process.

22.3.4.3 Nurse Staffing

The provision of recommended neonatal nurse staffing levels again will vary between health systems, depending on the roles that are undertaken by nurses and the availability of other professionals, such as respiratory therapists. Within the UK system there have been few studies of nursing input on which to base the recommendations set out in Table 22.2. Nonetheless it is apparent that adequacy of nurse staffing is a key issue within neonatal units in relation to mortality [15]. Further research is needed to support recommendations in this area.

In systems where nurses take on more technical roles (cannulation, intubation, ventilator management), enhanced nursing support is required. With appropriate training many units have developed the role of Advanced Nurse Practitioner; such individuals form an intermediate tier between junior and senior resident doctors and assume many of the roles previously taken on by medical staff (at least in the UK) and may contribute to on call rosters [16]. In some settings they may act without medical staff in supporting low risk deliveries, whereas in other settings they provide intensive care activity. Both these and other roles require different and bespoke training.

22.3.4.4 Medical Staffing

Medical staffing is equally dependent upon the health service model. In the UK, medical trainees and Advanced Neonatal Nurse Practitioners support neonatal intensive care services at a technical grade (junior resident) and as experienced senior resident support. The number of staff neonatologists in this system is relatively few and they have more of an executive and managerial role. Changes to working hours (for example the European Working Time Directive) and availability of resident staff to work in these high intensity settings mean that such systems are under threat. In many other settings, staff neonatologists (nationally-qualified doctors) provide medical care.

Research into the numbers of neonatologists required to staff a neonatal service is unusual. One epidemiologic approach determined that mortality rose in areas of the USA where there was a very low supply of neonatologists (<4.3 doctors per 10,000 births) but that increasing the number did not result in further reductions [17]. In the UK the number of consultants was not related to mortality or nosocomial infection rates [18].

22.3.4.5 Clinical Governance

Within any neonatal service a strong clinical governance framework is required in order to maximize safety and efficiency. Clinical governance comprises a range of organizational structures which we use as professionals in order to quality assure the care we provide, the most useful of which are:

- the clinical audit process
- the use of standards, guidelines and perinatal audit tools
- benchmarking and the application of evidence based care.

Although all three are interwoven they merit consideration separately.

Clinical Audit

The key to the process or cycle of audit is the setting of standards, the appraisal of your service against the standard and the revision of your standard consequent upon the audit, to be followed by a re-audit to make sure it is appropriate. The areas that are amenable to audit cover the range of activities of a neonatal service and include the structure of the service (people/facilities available); the process of care (are we consistently doing what we think we are?); and the outcome of care (Is our prophylactic indomethacin effective?; Is our mortality rate appropriate for our population?).

Clinical audit is a key part of assessing the service we are giving and audit of structure and outcome can provide valuable information when bidding for resources and re-designing services. It also helps to confirm that we are achieving what we aim to achieve in terms of outcomes and activity. More information on the use of clinical audit in neonatal care is to be found elsewhere.

Standards and Guidelines

Part of the way in which we develop our service is to provide a range of policy that is based around the best evidence we have. In the USA, the Fetus and Newborn Committee of the American Academy of Pediatrics produces regular guidance based on best evidence. In the UK, guidance on aspects of clinical care is sometimes available from professional organizations and may be specified as part of the process of resourcing the service. The series of Green-top guidelines produced by the Royal College of Obstetricians and Gynaecologists, for example, contain much of relevance to neonatal care and guide practice widely in the UK. The Royal College of Paediatrics and Child Health publishes a range of documents, which although not primarily aimed at neonatal care are of great importance in neonatal practice, for example, Withholding or Withdrawing Life Sustaining Treatment in Children: A Framework for Practice (2004), which guides practice around end-of-life decisions. The British Association of Perinatal Medicine (BAPM) also produces a range of practical guidance, for example:

- Management of extremely preterm babies (2008)
- Acute in-utero transfers (2008)
- Screening and treatment of retinopathy of prematurity (2008)
- Classification of Health Status at 2 years as a perinatal outcome (2008)
- Early care of the newborn (2005)
- Consent in neonatal clinical care (2004).

Further guidance is published on an ad hoc basis, for example, the recent European Guidance on the Management of Respiratory Distress Syndrome [19].

Most neonatal intensive care units are busy places and it is helpful to have a range of guidance easily assessable to staff. It is also helpful to work to protocols so that audit of care is more meaningful. Key aspects of guidelines are that they should:

- be evidence based
- explain in principle why a treatment/process is preferred
- be auditable to ensure they are working.

Within the UK neonatal networks, guidelines are usually shared for key areas of practice: referral, transfer and early care for very preterm babies, for example, and shared between units via their websites. Within the Trent Neonatal Network (www.trentperinatal.nhs.uk), for example, the following guidelines are available:

- Neonatal transport
- Surgical referral
- In-utero transfer
- Early care.

Benchmarking

Comparing the performance of one unit with another is fraught with difficulty. Population risks are different and can lead to erroneous conclusions (see Chapter 143).

Table 22.4 Recommended format for reporting perinatal outcomes at 2 years [21]

Gestational age at birth		22 w	23 w	…	31 w
Number of births (exclude terminations of pregnancy)[1]					
Number of live births					
Number of admissions for intensive care					
Number of survivors discharged home					
No. of deaths between discharge and 2y					
No. of survivors evaluated at 2y					
No. with cerebral palsy					
No. with motor impairment GMFCS 2 *					
No. with motor impairment GMFCS 3-5 **					
No. with Cognitive score <–2 SD *					
No. with Cognitive score <–3 SD **					
No. with hearing aids but not severe hearing impairment *					
No. with severe hearing impairment **					
No. with speech and language impairment *					
No. with severe speech and language disability **					
No. with visual impairment but not severe visual impairment *					
No. with severe visual impairment **					
Total with neurodevelopmental impairment (moderate or severe) *					
No. with moderate disability					
No. with severe neurodevelopmental disability (SND) **					
Moderate impairment	% survivors evaluated				
	% admissions for NIC				
	% births *				
Death or moderate impairment	% admissions for NIC				
	% births *				
Severe impairment	% survivors evaluated				
	% admissions for NIC				
	% births *				
Death or severe impairment	% admissions for NIC				
	% births *				
No. with other disability					
Describe					

[1] "Births" may be total births or particular groups (e.g., gestational age) in a defined population – this could be hospital based, network based or population based depending upon the need.
* Components of moderate disability – a child with an impairment in any category (but none in the severe category) is classified as having moderate disability.
** Components of severe disability – a child with any one severe disability or impairment in any category is classified as having severe impairment.
GMFCS Gross motor function classification system.

However, it is important to establish an external reference for the performance of a neonatal service and there are important resources available to support this – such as EuroNeoNet (www.euroneostat.org) and the Vermont Oxford Network (www.vtoxford.org), in addition to national or local benchmarking systems. Whereas the exact ranking in such datasets is not really helpful, progress against the reference population and the identification of areas where a service is an outlier are invaluable pieces of information. The most valuable benchmarking process in the UK is the national Centre for Maternal

and Child Enquiries (CMACE) process (formerly known as Confidential Enquiry into Maternal and Child Health (CEMACH) and Confidential Inquiry Into Stillbirth and Death in Infancy [CESDI]), whereby there is national monitoring of perinatal and maternal mortality with focused confidential enquiries and other audit projects (www.cmace.org.uk).

Alongside the benchmarking process, the Vermont Oxford Network offers a range of quality improvement programs, which serve to help a service deliver state of the art care and minimize morbidity, in areas such as nosocomial infection, chronic lung disease and intraventricular hemorrhage through a process of audit.

Data Collection

The key to all aspects of clinical governance is the collection of robust and accurate information about the unit and the babies it looks after. There is a range of datasets available but all require close attention to details to ensure that what is collected is accurate and useful. BAPM has produced a UK national dataset [20] and the international benchmarking groups require similar datasets. The recent National Health Service Neonatal Taskforce has recently published a series of Principles for Quality Neonatal Care that will form the basis for development of the UK dataset further [11].

22.3.5 Follow-up after Discharge

Most neonatal services now provide follow-up for groups who are at high risk of disability. In the UK, a recent working party has revisited the criteria for defining health status as an outcome measure for perinatal care, recommending a formal assessment and recoding of data at 2 years of age [21]. These are similar to those used within the The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Neonatal Network in North America. Target groups for follow-up are very preterm or very low birth weight children, children who have been encephalopathic and any that are identified by other routes to be at risk. Combined with mortality data, this powerful information is useful in terms of monitoring outcomes and providing centre based information for parents. An example of the output from such a service is shown in Table 22.4.

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23

Training of Doctors and Nurses in Perinatology

Franco Macagno and Alfred Tenore

A consideration and comparison of the training of neonatologists in developing and developed countries would be beyond the scope of this chapter because the vast differences which exist would compile enough material to be a book in itself. The reason for this, as one would expect, is that there is hardly anything which may be referred to as a unifying standard of training in developing countries [1]. Unfortunately what is more un-consoling is that there is no unifying standard in developed countries as well.

23.1 Comparison of Some Exemplary Training Systems

The educational track to a career in neonatology in the United States, regardless of the State where the training takes place, goes through an initial three year pediatric training program, or "residency", comprised of time in the clinic, inpatient wards, and emergency department under the guidance of pediatric faculty. During this training, the resident takes care of patients in a closely supervised environment, goes to daily lectures and teaching conferences, and works night shifts to handle pediatric emergencies in the hospital. The resident is also exposed to a broad range of pediatric subspecialties (including pediatric and neonatal intensive care). Subsequent to pediatric training, an additional 3 years of training called a "neonatal fellowship" is required to become a neonatologist. This time is typically divided between taking care of sick babies in a neonatal intensive care unit, under the constant supervision of experienced neonatologists, and usually, clinical or basic science research. The neonatology trainee is required to learn to handle the full spectrum of neonatal problems and diseases as well as writing an article about a research project related to newborn care.

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One big difference between United States and united Europe, is that Europe, in many things, particularly medical and specialty training, is not yet "united". The same can be said for an attempt to compare programs throughout the world. It is for this very reason that one of the major concerns of the UEMS (European Union of Medical Specialists), since its creation in 1958, has been to harmonize the different training programs for the different specialties in order to ensure the highest possible health care for European citizens. Unfortunately, after 50 years this still has not been achieved even though assuring the highest and uniform possible health care for its citizens should be of primary and vital importance.

Needless to say, the formation of the European Union (EU) has had many positive political and economic consequences through the enforcing of *standards* which member states are required to follow. However, it has also allowed many problems to surface, especially in the field of medicine where, paradoxically, no such standards have been enforced even though EU guarantees the recognition of medical education and training programs as valid in all member states as well as the free movement of physicians throughout Europe. This European directive allows doctors to work wherever they like, even though training programs in Europe both at the medical or specialist graduate level are not comparable in quality or content. In order to achieve high standards of patient care, high quality post-graduate training programs are indispensable. The European Academy of Paediatrics (EAP), the Paediatric Section of UEMS, along with the European Board of Paediatrics, a standing committee of the EAP, has the specific objectives to standardize training throughout EU.

The European example is only one of many that exist throughout the world which reveal great variations in training. In fact, key indicators of health, such as neonatal, infant and under 5 yrs mortality rates as well as disease morbidity, reveal dramatic differences between children living in developed and developing nations. Although many socio-economical factors are responsible for these differences, an important, although rarely emphasized disparity in quality of life for children, is the lack of access to trained pediatricians, let alone neonatologists.

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Pediatric training in developing countries can range from well-organized programs to very informal ones; however, even where standards for training have been delineated, these alone are frequently inadequate for a variety of reasons (Chapter 22).

Neonatology is a highly technical and rapidly evolving area of pediatric medicine. In the USA, most but not all neonatologists are *board certified* in the specialty of pediatrics by the American Board of Pediatrics (ABP), and in the sub-specialty of neonatal-perinatal medicine also by the ABP. Most countries now run similar programs for fellowship training in neonatology. A doctorate of medicine in neonatology, referred to as a DM (Neonatology), from India is one such highly regarded program. In most EU countries, like in the USA, neonatology is established as an independent specialty following post-core training in pediatrics. Although it is emphasized that the curriculum be delivered over a minimum of two years it is highly recommend that individual EU countries move to deliver this curriculum over three years in the interest of training competent practitioners in neonatal medicine and to provide consistency. However, the form and duration of training, as well as the process for accrediting training centers and monitoring the quality of training, vary markedly from country to country.

23.2 Recommendations for Appropriate Training Programs in Neonatology

The amount of time necessary to form a competent neonatologist is somewhat difficult to standardize because it depends on a variety of factors among which the degree of expertise and the necessary material educational resources available. Because of this fact, the emphasis must be placed on establishing clearly defined standards of training aimed to equip the pediatrician with the necessary knowledge, skills and attitudes required to practice high quality neonatal medicine. Appropriate *formation* requires the acceptance of a standardized *core curriculum* elaborated by some of the world's leaders in neonatal education. The essential elements of the core curriculum include (a) a "syllabus", or a detailed listing of the knowledge that needs to be covered during training, (b) a number of clinical skills and procedures that need to be mastered during training, and (c) an "accredited" structure where the training will take place.

The trainee neonatologist should acquire detailed knowledge in the following categories: (a) epidemiology, (b) patho-

Table 23.1 Knowledge-based content of core curriculum

Epidemiology	Mortality and morbidity rates in the perinatal period and factors which influence mortality and morbidity; methods of data collection, including birth and death notification systems and audits aimed at quality assessment.
Pathophysiology of the fetus	Fetal growth and development and the means of its assessment; impact of the major diseases of pregnancy on the fetus (i.e. hypertensive disease, maternal medical conditions, antepartum hemorrhage, and preterm labor); detection of fetal anomalies and collaborative prenatal counseling.
Physiology of postnatal adaptation	Respiratory, cardiovascular and other physiological changes at birth; development of organ systems and physiological changes after birth; physiology of breastfeeding.
Pathophysiology of prematurity	Respiratory development and pathology including surfactant deficiency and its sequelae; cardiovascular problems including patent ductus arteriosus and persisting pulmonary hypertension; gastrointestinal development and feeding, renal maturation and fluid balance; neurological problems, including pathogenesis of intraventricular hemorrhage and periventricular leukomalacia.
Pathophysiology of conditions encountered in premature and mature infants	Congenital abnormalities and their management; perinatal hypoxia and consequences of hypoxia and ischemia; metabolic adaptation to postnatal life; inborn errors of metabolism including screening programs for their detection; neonatal immunity and pathogenesis of perinatal/neonatal infection.
Pharmacology in the perinatal/neonatal period	Pharmacokinetics in the term and preterm newborn; drug toxicity and interactions; influence and effects of maternal medications and drug abuse on the fetus and newborn infant; transmission of drugs via breast milk.
Principles of neonatal care	Theory and organization of resuscitation; respiratory care and mechanical ventilation, endotracheal intubation and delivery of respiratory support; management of complications and long-term sequelae of prolonged neonatal ventilation; cardiovascular support, assessment of cardiovascular system and of patent arterial duct; postnatal growth, breastfeeding, composition and use of neonatal formulae and supplements; parenteral nutrition, prescription, administration and indications; assessment, diagnosis and management of severe enteral diseases; neonatal skin and thermal care; assessment of fluid balance and nutritional requirements; assessment of bone mineralization; assessment of structural and functional integrity of the brain using clinical examination and special investigations; prognosis of major neuropathology, screening preterm and "at risk" babies for retinopathy and hearing loss; diagnosis and assessment of congenital abnormality and dysmorphology; investigation of suspected inborn errors of metabolism; use of genetic investigations and diagnostic aids; routine care of the newborn in relation to jaundice, breastfeeding, infections; screening for neonatal disease by examination and investigation: early, medium term and late sequelae of neonatal and perinatal events and ethical issues in neonatal care.
Follow-up of high risk infants	Outcomes associated with perinatal high risk groups, e.g., prematurity, fetal growth restriction and intrapartum hypoxia; diagnosis and counseling associated with cerebral palsy, visual and hearing defects, chronic respiratory problems and an understanding of the importance of other neurocognitive outcomes.
Ethical issues and legal problems	

Table 23.2 Skills-based content of core curriculum

Practical procedures	Resuscitation of the newborn, tracheal intubation and techniques of artificial ventilation; insertion of umbilical and peripheral arterial catheters; establishment of intravenous infusion and long intravenous lines; blood transfusion and exchange transfusion; arterial puncture, pleural drainage of pneumothorax, suprapubic aspiration of urine, lumbar and ventricular puncture.
Diagnosis	Interpretation of neonatal chest and abdominal radiological investigations; role of specialized investigations, e.g., MRI, CT; experience in interpreting results of ultrasound examination of the nervous system, the abdominal organs, and of congenital hip dysplasia; ordering and interpretation of common laboratory and microbiological investigations; use and interpretation of the results of EEG, cortical evoked responses and neuromuscular electro-physiological tests.
Clinical practice	Clinical examination of sick and healthy newborn babies; recognition of specific neonatal problems including deformation and malformation; assessment of gestational age; developmental and neurological assessment of the older infant and child and the assessment of disability.
Communication	Counseling and communication skills including appropriate approach to distressed and bereaved parents, disclosure of "bad news", handling of autopsy reports; staff support and team dynamics; co-operation and consultation with other medical specialists.
Technology	Understanding basic mechanical and electrical function of radiant heaters, incubators, ventilators, and monitoring equipment.
Teaching	Training and involvement in teaching activities including teaching programs for doctors and nurses.

physiology of the fetus, (c) physiology of postnatal adaptation, (d) pathophysiology of prematurity, (e) pathophysiology of various conditions encountered in premature and mature infants, (f) pharmacology in the perinatal/neonatal period, (g) principles of neonatal care, (h) follow-up of high risk infants and (i) ethical issues and legal problems. Table 23.1 reports a more detailed list of the major topics of each of the points listed above and which represent the knowledge-based content of the core curriculum.

Trainees will also be expected to have acquired extensive skills in the following domains: (a) practical procedures, (b) diagnostic related studies, (c) clinical practice, (d) communication, (e) technology and (f) teaching (Table 23.2). In addition, Table 23.3 describes competencies which may be considered key elements in the training of the neonatologist and the efficient and competent practice of neonatology. The neonatal specialist's role also includes emphasis on personal development in leadership within the clinical team.

Furthermore, since today's neonatologists undertake important management roles within the team and within their host organization (usually their Hospital or University), training programs must also equip the trainee with the personal skills necessary to fulfill these roles, which include: (a) counselor, (b) manager, (c) leader and (d) teacher. In addition, the trainee must also have acquired experience in clinical governance and audit as well as in the field of statistics where he should be skilled to appropriately interpret statistical data.

Worldwide, about 98% of neonatal deaths occur in developing countries where maternal risk during pregnancy and at delivery is very high. Major improvements in neonatal and infant mortality and quality of life can be achieved by reducing causative factors. In these areas, the application of a regional organization, comprising a three level perinatal service, may not be considered the main priority. Furthermore, the establishment of a network coordinated by a regional center may also be difficult because of unfavorable geography, which will add to the problems of establishing an efficient transport system that ensures the timely and safe transport of the pregnant woman and high risk newborn [2]. In these situations, maternal and neonatal care receive minimal government funding. Furthermore, thousands of first level hospitals and improved neonatal intensive care units would be required. Basic problems should first be addressed and training of personnel attending pregnant women and neonates should be the main priority. Great distances between regional centers constitute a problem not only for maternal and neonatal transport but also for the education of physicians, nurses and midwives.

In most areas, the main educational aim should be good quality home-based neonatal care post hospital discharge. Midwives and caregivers should be trained to identify normal and sick neonates, to resuscitate hypoxic newborns, to support breastfeeding, to prevent and treat neonatal infections, to satisfy the basic needs of preterm babies and to recognize malformations.

Perinatal care programs based on a three level organization should only be considered when mortality in a region falls below 15/1000 live newborns. The effect of local differences is that there is no single program or strategy that can be universally applied to developing countries. Decisions should be made on the basis of the local background, economic resources, number and educational status of personnel, geographic, social and demographic conditions.

In developing countries with high neonatal morbidity and mortality, efforts should be directed at improving primary care models. In order to strengthen primary care services, an education program aimed particularly at midwives is effective in reducing neonatal mortality rate. Thus, each region should establish a program that is appropriate to its own requirements, depending on resources, local conditions, personnel and the availability of outreach education [3].

Table 23.3 Key competencies in neonatology that trainees must be capable of demonstrating

Resuscitation	Be able to institute and lead neonatal resuscitation both of the term and preterm baby and demonstrate a full understanding of the physiology and treatments involved.
Neurology	Proficiency at clinical assessment; investigation, including cerebral ultrasound scanning, and management of a range of neurological disorders, including preterm and term brain injury, congenital malformations, intracranial trauma and seizures.
Communication skills and counseling	Increasing skills in communication with parents and staff, both individually and as part of a team, during their training; this includes experience at breaking bad news, handling perinatal death and discussing prognosis with parents.
Congenital anomalies and genetic disease	Able to recognize common congenital anomalies, to investigate babies with such lesions and to use literature and database searches to identify rare conditions and communicate such information to parents.
Cardiorespiratory intensive care	Be able to plan for the care of the baby with chronic respiratory disease and be aware of the potential long-term complications. Be able to institute and maintain full cardiorespiratory intensive care for preterm and sick term newborn babies demonstrating a full working knowledge of the principles and application of a range of ventilatory modalities and of circulatory support.
Fluid balance, thermoregulation and renal failure	Be able to initiate and manage the thermal environment of preterm and term babies, and manage fluid balance in such babies, demonstrating a full understanding and knowledge of the underlying physiology – with special reference to the rapid postnatal changes in body water distribution and transepidermal water loss. The trainee should be able to diagnose and initiate treatment of renal failure.
Hematology and transfusion	Be able to diagnose and manage the range of hematological disorders found in newborn babies and be conversant with the full range of blood products available for transfusion and the appropriate use of each.
Metabolism and endocrine disorders	Proficiency in the recognition, assessment, investigation and management of the more common and important metabolic and endocrine disorders.
Nutrition, feeding, gastrointestinal and hepatic disease	Understanding of the importance and principles of neonatal nutrition and be able to provide comprehensive nutritional support to well and sick newborn babies, including the recognition and treatment of common complications; be able to recognize both common congenital gastrointestinal and hepatic anomalies and acquired neonatal disease.
Immunity and infection	Understanding the development of immunity and the vulnerability of the newborn to infection.
Family care and care of the well newborn baby	Acquisition of a wide knowledge of normal development, common minor problems and morphological variation and the importance of communication with other health care professionals and the parents.
Transport of the newborn baby	Be competent at retrieval and transport of the sick newborn baby and be able to teach others to carry out transfers.

23.3 Conclusions

Given the enormous diversity that exists throughout the world in the formation of a neonatologist related to the duration of the training as well as the structure of the training program, the object of this chapter was not to attempt to compare the various programs but to give information regarding the common knowledge, skills and competencies that are needed to be acquired by a trained pediatrician in order to become a neonatologist. The EAP has published a syllabus for trainees in neonatal medicine which details the range of subjects that trainees should cover during the neonatal sub-specialist training. The syllabus can be downloaded from the EAP website (http://www.EAPaediatrics.eu). However, since knowledge, skill acquisition, training, and general competence occur in a continuum of lifelong learning, trained neonatologists should be committed to a continual state of learning and evaluation in order to remain up-to-date with advances in medicine and to provide evidence to the public that they remain competent to practice their specialty [4, 5].

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24

Neonatal Transport Services

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24.1 Introduction

Neonatal transfer is necessary when a newborn baby needs care that cannot be provided in the referral centre. Since it represents an additional risk factor for a critically ill neonate [1, 2], it should be performed, when possible, by a well-organized neonatal transport service.

In a network, aimed at the regionalization of perinatal care, high-risk pregnancies should be transferred "in utero" in order to minimize risks for both mother and neonate [1, 3]. However, there will always be a number of neonates who need to be transferred for unpredicted or unpredictable reasons [4]. Every maternity unit should therefore be able to provide effective neonatal resuscitation in the delivery room, be able to maintain a sick baby in a stable condition in the short-term and have access to a Neonatal Emergency Transport Service (NETS) for the provision of neonatal intensive care when appropriate. Thus, NETS provides a bridge between birth centers and Neonatal Intensive Care Units (NICUs).

24.2 NETS: a Mission Statement

NETS should aim to guarantee:

- optimal care for every neonate in the most appropriate location
- safe and quick transport with minimization of risk
- good communication between transport team, parents and the centers involved
- rational resource use with optimized cost-benefit ratio
- an outreach education program relating to resuscitation and pre-transport stabilization
- guidelines for referral centers and transport personnel.

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The establishment of a NETS therefore requires planning and organization. There should be regular auditing to monitor its efficiency and effectiveness (see Chapter 22).

24.3 Organization

The organization of a NETS should be appropriate to the needs and characteristics of the region covered and resources available. Account should be taken of:

- demography (birth rate and distribution)
- place, number and levels of perinatal units
- number of transports per year and patient flow between centers
- neonatal transport index (NTI, see below)
- available economic resources, personnel, vehicles and equipment
- regular clinical audits.

There are different models of perinatal networks for the provision of the most appropriate service. The choice between the two models in terms of cost-benefit ratio depends mainly on the number of transports per year and on the referral area [6], as follows.

- *Dedicated service*: all transports carried out by a team devoted full-time to this service. The optimal number of transports per year is 400-600. Fewer than 400 increases costs and more than 600 decreases efficiency.
- *On-call service*: in this organizational model, transport is integrated into the overall activity of an individual NICU and carried out by "in-house" personnel. The optimal number of transports per year to minimize costs and maximize efficiency is 150-200.

The characteristics of each model are summarized in Table 24.1. In Europe, the most usual models for regional networks are dedicated or on-call NETS [5].

The NTI is an expression of the number of neonates transferred per 100 live births. This index can be used to compare the flow of newborns between facilities in each area. A low

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Table 24.1 Characteristics of the two NETS models

	Dedicated	On-call
Activation times	Usually shorter	Usually longer
Availability	Around the clock	Available on-call
Distances covered	Longer	Shorter
Area covered	Usually larger	Usually smaller
Receiving centre	All NICUs (interfacility transports)	Only NICU where NETS is based
Individual specific transport expertise	More, because mainly appointed to transport service	Less, because temporarily detached from NICU
Costs	More expensive	Less expensive

NTI (around 1%) indicates a good distribution of perinatal facilities and well-organized maternal-neonatal transport services. Where high-risk perinatal care has been regionalized, as in the UK, the NTI is about 1% [7, 8], whereas in areas with heterogeneous distribution of obstetric units, the NTI can reach 10%, e.g. the Loire–Atlantique region in France [9].

To account for regional diversity, it is also possible to organize a network with more than one type of NETS.

24.3.1 NETS Coordinating Centre

Each regional network should ideally have a NETS coordinating centre. Its role is to:

- triage requests for transfers
- monitor cot availability and find appropriate cots
- activate the appropriate transport team and co-ordinate the transfer
- prioritize simultaneous requests
- give advice to referring clinicians and to the transport team
- supervise staff, plan audits and education
- ensure the provision of appropriate resources.

24.4 Types of Transfers

Transfers can be classified on basis of the degree of emergency and destination:

- *acute*
  - primary transfers: to a NICU or a higher level centre, depending on the requirement for intensive or high dependency care;
  - intertertiary: between NICUs, if there is a lack of cots or the need for surgical or specialized care;
- *elective*
  - back-transports: usually from a NICU to the local hospital, when a neonate is stable, to maintain the availability NICU cots, and minimize costs and discomfort for families [10];
  - return transfers: for diagnostic investigations (e.g. MRI) or procedures (laser treatment for ROP).

24.5 Transport Process

The transport process (Table 24.2) starts with a request for transfer by the referring centre. Each transport should be coordinated by the coordinating centre, or by a senior neonatologist at the admitting center.

24.5.1 Patient Stabilization

It is important that staff at the referring hospital should be able to perform basic resuscitation and stabilization before the arrival of the transport team. The baby's clinical condition should be carefully evaluated to establish the initial diagnosis and a therapeutic plan. Stabilization involves basic physiological support and is defined as the treatment or correction of those processes which, if not addressed, may lead to deterioration in the baby's condition. After stabilization, preparations for transfer are started. These include ensuring that physiological parameters are satisfactory, that airway and vascular access are secure and that appropriate monitoring is in place. Insufficient attention to resuscitation and stabilization may result in clinical deterioration during the transfer and the environment during transport (limited space, vibration, noise, etc) is not conducive to optimal patient care. Good communication between team members and between the team and the referring staff and the patient's family is essential.

24.5.2 Personnel

The choice of personnel for a transport team varies widely between NETS, depending on organizational preferences, budgets and availability of professional groups [6, 11]. The traditional view that a neonatologist and a nurse should constitute a neonatal transport team is no longer generally accepted. Retrieval teams with different professional backgrounds do not appear to affect the outcome of transports [12, 13].

The nature of the professional background of transport team members is less important than training in transport practice with specific core competencies [14]. Poor transport

Table 24.2 Transport process

Events	Operative approach
Request for transfer	Discussion, triage, advice and support for referring centre, plan of action
Cot search	Finding the most appropriate cot and activation of the transport team
Transport team departs from base hospital	Continuing advice and support for the referring centre, which is responsible for pre-transport stabilization
Transport team at the referring hospital	Assessment and further stabilization prior to transport
Transfer of the baby to admitting hospital	Monitoring, continuous assessment, specific treatment if needed
Transport team at the admitting hospital	Final assessment, handover to admitting team
Transport team return to base hospital	Check and replacement of used equipment and medical gases, communicate new availability

experience reduces quality of NETS [15, 16]. Time devoted to stabilization of the neonate prior to transport increases with operator experience [17].

There should be regular rotation of personnel between NICU and the transport service to prevent burnout and allow for professional updating.

24.5.3 Equipment

Equipment taken on transfer needs to be suitable for a variety of babies, from the extremely low birth weight to term, and for the range of medical or surgical problems that require different therapies in transit. The transport itself requires a transport incubator system with a mechanical ventilator, infusion pumps, suction pump and monitors. A neonatal resuscitation kit, including drugs should be carried in a separate bag.

Equipment should be dedicated exclusively for neonatal transport, to ensure its ready availability and that the battery is fully charged. Some recommendations have been provided by the European Committee for Standardization [18, 19]. Difficult working conditions and mechanical stresses, caused by vibrations and sudden forceful impact, mean that equipment should be checked and tested regularly. Staff should be trained in their use and should be aware of the more frequent minor causes of malfunctioning so they are able to fix them.

24.5.4 Road and Vehicles

The choice of road and vehicle should aim to guarantee the quickest, safest and most comfortable transport for the baby, taking account of clinical conditions, regional geography, weather, road and traffic conditions [20]. Different scenarios should be considered and guidelines prepared to determine the choice of vehicle and appropriate response to emergency situations. Vehicles that can be used by NETS are ambulance, helicopter and fixed wing aircraft. Ambulances are most commonly used because of lower costs, universal availability, short activation times and the possibility of reaching hospitals directly. Dedicated vehicles guarantee shorter activation times and better compatibility between equipment.

Air transport, including by helicopter, is indicated for longer distances and for regions with poor weather conditions during winter or peculiar geographic characteristics (e.g. mountain regions). Account should also be taken of the possible need for an additional ambulance journey, depending on whether there are facilities for landing at the hospital. Air transport is characterized by the presence of high noise and vibration levels, changing of gas pressures, temperatures, humidity and gravitational forces that may affect care and possibly worsen the condition of the critically ill neonate and, for these reasons, should only be performed by specifically trained personnel [21].

24.6 Quality Evaluation and Costs

Audit is essential for the evaluation and improvement of the service [22, 23]. Data collection should include demographics, staffing and pre- and intra- transport times, as well as clinical parameters.

NETS availability and time required can be considered as indicators of efficiency [24]. Time investment includes:

1. the response time (period between call for transport and departure for referring hospital);
2. the waiting time (time between call for transport and arrival at referring hospital);
3. the stabilization time (time required to assist neonate at referring hospital);
4. "on the road" time (time with baby in transport between departure from referring unit to admission at receiving hospital);
5. the total transfer time (from departure towards referring hospital to availability of the team for another transfer).

Measures of effectiveness can be based on changes in the infant's core temperature during transfer, validated transport scores [25, 26, 27] and monitoring complications during transport.

The annual budget for running a NETS includes fixed and variable costs. Fixed costs are independent of the number of the transports and include equipment amortization, insurance cover, training and education, organization and communication. Variable costs depend on the number of transports and include supplies, equipment maintenance, and ambulance

expenses. Personnel salaries depend on the type of NETS: in the "dedicated" model, this is a fixed cost, whereas in the "on call" model it affects both fixed costs (depending on availability) and variable costs (actual hours). The total cost of the NETS and the average cost of each transport depend on the fixed and variable costs, which in turn depend on the organizational model adopted and the load of scheduled jobs [6].

24.7 Training and Outreach Education

Continued improvement of perinatal outcomes depends on all health professionals recognizing their responsibility to keep up-to-date with recent advances in perinatal care, by updating their knowledge and clinical skills.

Transport personnel must receive appropriate training and supervision. Core competencies include clinical, procedural, communication and teamwork skills, and the ability to work in the transport environment [14].

Continuing education in neonatal transport involves training and supervision of all members of the transport team. It relates not only to clinical care but also to safety and communication, as well as to administrative and other relevant topics. Extending continuing education throughout the referral region (outreach education) is also important for the healthcare professionals responsible for referring and stabilizing infants for transport [6].

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25

Problems of Discharge and Home Care of Newborns

Fabio Mosca and Monica Fumagalli

Improved obstetric and neonatal care has increased the survival of very preterm babies and very ill newborns who are often discharged with unresolved medical problems or special health care needs. A close follow-up and an individualized home-care plan have to be developed for these babies before discharge from NICU.

High-risk newborns include premature infants, babies requiring special care and technology dependent infants, defined as those needing ventilatory or nutritional support.

25.1 Timing of Home Discharge

According to the guidelines of the American Academy of Pediatrics [1] timing of home discharge should be based on physiologic criteria rather than body weight.

Physiologic stability and the acquisition of the following competencies are considered essential to allow home discharge: 1) respiratory control; 2) thermal stability in home environment; 3) competent oral feeding (by breast or bottle) for an appropriate growth.

25.1.1 Respiratory Control

Significant apneas of prematurity can persist up to 43 weeks' postmenstrual age (PMA). After stopping methylxanthines administration, babies who have experienced problematic apneas should have an event-free period of 5–7 days before discharge. It is known that the prone position improves oxygenation, while the nonprone position decreases the incidence of sudden infant death syndrome (SIDS) [2, 3]. The association between prone sleeping and SIDS among low birth weight infants is equal to or even stronger than the association among those born at term. Therefore, prior to discharge, babies should spend a period of weeks in the supine position monitoring the occurrence of desaturation events and mothers should be strongly advised by caregivers during hospitalization to maintain supine sleeping position when at home.

25.1.2 Immunization

Preterm infants should receive routine recommended vaccines at a chronological age consistent with the schedule recommended for full-term infants [4].

During the respiratory syncytial virus (RSV) season palivizumab should be given before discharge to high-risk neonates according to the national recommendations [5].

25.1.3 Neonatal Screening Programs

Newborn metabolic screening should be completed and reviewed before discharge. Research data have shown that infants in the NICU are at highest risk of having neural hearing loss. Therefore, a hearing test should be performed before discharge in high-risk babies and a long-term careful monitoring for hearing loss, with subsequent appropriate audiological management, is essential after discharge.

25.1.4 Parental Education

An active program of parental involvement in the infant's care should be established by caregivers as soon as possible during the hospital stay in order to teach parents to handle the

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

baby and let them acquire skills and competence needed to take care of their infant at home.

Breast milk should be strongly promoted as the optimal diet also for high-risk infants and soon after birth mothers should be encouraged to provide milk for their own infants. After discharge, mothers should be supported to continue breastfeeding or to give their own expressed milk; considerable psychological benefits of breastfeeding have been also described.

Before discharge a planned follow-up program should be offered to the parents by an identified primary care physician who will take the responsibility of the baby's care after discharge. Individualized discharge and follow-up plans are important to assure safe and effective care at home and to minimize avoidable hospital readmissions. In order to help parents to cope with the baby's needs at home additional support may be provided by home nursing care.

25.2 Special Care Needs

25.2.1 Ventilatory Support

Babies with chronic lung disease (CLD) may require long-term oxygen supplementation to maintain oxygen saturation at an acceptable level and avoid growth failure. In order to shorten hospital stay home oxygen therapy should be considered for these babies with increased inspired oxygen need. Some infants may require oxygen supplementation for months or even years and weaning from supplemental oxygen should be gradual to avoid worsening of pulmonary hypertension.

For infants who cannot be weaned from assisted ventilation or infants with upper airway abnormalities home ventilation may be necessary. In this case a tracheostomy has to be performed before discharge and a special education for parents and qualified home care are needed.

High-risk newborns may require pulse oximetry or apnea monitoring after discharge. Although criteria to home apnea monitoring vary widely among NICUs, recommendations for home monitoring include babies presenting mild episodes of apnea not requiring stimulation, having a feeding tube in situ and requiring oxygen supplementation or ventilatory assistance.

The use of home monitoring is not indicated to prematurely discharge babies who do not demonstrate maturity of respiratory control.

25.2.2 Nutritional Support

Competent breast or bottle feeding supporting appropriate growth is advocated before discharge. Nevertheless, the use of a feeding tube at home may be considered when feeding is the last issue precluding home discharge. A safe home management of feeding tube can be assessed for a limited time period by well educated parents; when the baby does not improve in oral feeding skills a gastrostomy should be performed to guarantee safe and effective gastric nutrition. During gastrostomy feeding oral nutrition must be stimulated as removal of naso/orogastric tube reduces discomfort during suction and may improve oral feeding skills.

Infants with short bowel syndrome due to pre- or postnatal damage of the gut usually require total parenteral nutrition (TPN) when less than 40 cm of small intestine remains. If TPN is likely to be needed long-term, home administration has to be planned to give these children a better quality of life. Home TPN requires medical devices and intense home nursing for surveillance of central venous catheter related problems until parents achieve competency and independency.

25.3 Late Preterm Infants

Infants born at 34+0 through 36+6 weeks gestation were previously referred to as "near-term" babies. A change in terminology from "near-term" to "late-preterm" is now recommended based on the evidence that these infants are less physiologically and metabolically mature than term infants.

The population of "late preterm" is at increased risk of developing medical complications during hospital stay and has a higher rate of hospital readmission during neonatal period (for jaundice, feeding difficulties, dehydration and suspected sepsis) [6] and later in infancy.

In a large national US study 15.2% of late-preterm infants were rehospitalized in the first year of age, compared to 7.9% of term infants [7].

Moreover, late preterm infants seem to be more likely to have a diagnosis of developmental delay within the first 3 years of life and to be referred for special needs preschool resources [8]. This observation highlights the importance of understanding the inherent risk of this subgroup of patients in order to design appropriate discharge planning.

25.4 Rehospitalization

Preterm infants have a high rate of readmission in the first year of life (about 15%), mainly due to respiratory illness, with the highest incidence in babies <25 weeks' gestation (31%), with CLD, living with other children, first discharged during the RSV season or belonging to socially disadvantaged groups [9]. The identification of associated risk factors can be useful to define high-risk groups of babies at discharge needing close follow-up and surveillance.

25.5 Safe Transportation

When positioned in a safety car seat premature babies and low birth weight infants have an increased risk of airway obstruction [10]. The potential risk of respiratory compromise is strictly related to anthropometric parameters (weight and length) and neurologic maturity as well as to the associated medical conditions, especially CLD. A car seat evaluation should be performed before discharge in newborns at risk for obstructive apnea, including babies with hypotonia (related to genetic or neuromuscular disorders), micrognathia or previous cardiac surgery. Parental education about the proper infant's position in the car seat is necessary to minimize the risk of respiratory impairment. A car bed may be indicated for infants who present apnea or decrease in oxygen saturation when positioned semi-reclined, unless evidence is not clear.

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26

Risk Management

Isabelle Ligi, Sophie Tardieu, Véronique Millet and Umberto Simeoni

26.1 Introduction

Rapid advances in neonatal medicine have caused a substantial reduction in neonatal mortality, especially in low birth weight babies. However, caring for newborn infants in the neonatal intensive care unit (NICU) exposes them to invasive therapies and therefore to iatrogenic damage. Moreover, NICU is a complex system with an environment of continuous and emergency care prone to error. Iatrogenesis is defined as an unintended harm or suffering arising from any aspect of health care management [1]. The report *To Err is Human* by the Institute of Medicine revealed the importance of medical errors in terms of patient harm, death and cost in USA [2].

Despite the fact that newborn infants constitute a high-risk population, data are still scarce. Incidence of iatrogenesis varies strongly between studies according to the methods of data collection and the definition used but recent prospective studies have reported high incidences of 0.4 to 0.74 iatrogenic events (IEs) per patient [3–6]. Many iatrogenic events result in permanent harm and more than a third are considered to be preventable [3–5]. They have huge financial, human and medico legal costs for the patient, their families and the staff [2, 7].

In response to a focus on improving patient safety, the concepts and practice of risk management have evolved during the last decade. Clinical risk management can be defined as organizational systems or processes that aim to improve the quality of health care and maintain safe systems of care [8]. It is based on a system approach, rather than an individual approach to error, taking account that human beings are fallible and that IE occurrence is a consequence of a successive lost of protective defenses, barriers and safeguards [9]. That is why risk management is an ongoing process based on an attitude (awareness of potential risk and liability), the knowledge and understanding of hazards, skills and commitment.

26.2 Epidemiology of Iatrogenesis in Neonatology

26.2.1 Definitions of Patient Safety Terms

Many definitions are commonly used in the patient safety literature. Using precise definitions helps interpret the findings of studies, as there is no standardized nomenclature.
Error: a failure of a planned action to be completed as intended or use of the wrong plan to achieve an aim [2].
Serious medical error: a medical error that could harm or injure a vulnerable patient if it reached the patient uninterrupted [10].
Adverse (medical) event: an injury caused by medical management rather than the underlying condition of the patient [2].
IE: any event that compromises the safety of the patient in the presence or the absence of harm. It may or may not be preventable and may or may not involve an error by the health-care team [3].
Critical incident: any event which could reduce, or does reduce, the safety margin for the patient [6].
Severe IE: any unintended injury or complication that results in disability, death, or an extended hospital stay, caused by health-care management.
Preventable IE: any avoidable iatrogenic event based on available knowledge and accepted practices [11].
A near-miss event: an event that could result in an injury, fatality, or property damage if not prevented.
Adverse drug event: any injury resulting from medical intervention related to a drug.
Medication error (ME): any preventable event that occurs in the process of ordering, transcribing, dispensing, administering, or monitoring a drug, irrespective of whether an injury occurred or the potential for injury was present [12].

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26.2.2 General Epidemiology of Iatrogenesis: Frequency, Severity, Preventability and Type

Data on epidemiology of iatrogenesis in neonatology are scarce and heterogeneous depending of definitions and methodology used. Table 26.1 shows data of seven reviewed studies. Undoubtedly, retrospective chart reviews broadly underestimate rates of iatrogenesis, with the estimate being that medical errors affect between 1.2 and 1.5% of admitted neonates [13, 14]. Actually, recent studies highlight the importance of iatrogenesis among neonates, as expected, in terms of frequency and severity. These studies (most of them based on a prospective, anonymous, voluntary and non-punitive system) reported high incidences of 0.4 to 1.25 events per patient [3–6, 15]. Two studies reported rates of 20 to 25 IEs‰ patient-days [3, 5]. As 30 to 50% of the incidents were judged to be severe and harmful, the impact on patient morbidity is considerable [3–6]. Preventability is often high from 35 to 83% of events [3–5].

Frequency of incident by category is heterogeneous, varying with local environments and patterns of problems. Nosocomial infections and respiratory events are reported to be common and often severe events. Particularly, unplanned extubations are a frequent (1.5 to 4.8 per 100 ventilation days), severe but largely preventable incident [16]. MEs and cutaneous injuries (Fig. 26.1) occur frequently in NICU but are often minor. ME rates vary widely between studies, partly due to differences in the definitions of an error and the methodology used for their identification. Kaushal and colleagues found an incidence as high as 5.7 MEs per 100 NICU prescriptions (91 MEs per 100 admissions) but included near miss events [12]. With near miss events excluded, Raju and colleagues reported a rate of 15 MEs per 100 admissions [17]. Dose errors, occurring during the prescribing and particularly the administration stage, are reported to be the most common type [18]. Ten-fold MEs are common and mainly related to errors in programming of flow rates of electric infusion pumps. Lastly, cutaneous injuries were found to be a frequent incident. Although skin necrosis was rare, scars constituted a severe and significant event with esthetic and sometimes functional troubles.

Table 26.1 Epidemiology of iatrogenesis in neonatal intensive care

Reference	Study design	Population	Characteristics of reporting	Definition	Results		
					Incidence/n reports	Severe	Preventable
Frey et al Switzerland 2000 [6]	Prospective 1 year	467 admissions (56% neonates)	Comprehensive, anonymous, non punitive	Critical incident	0.45 CI per admission	30%	Not described
Suresh et al USA 2004 [21]	Prospective Period 1: 17 months Period 2: 10 months	54 NICU	Non punitive, anonymous, voluntary, externally, internet based, free text (period 1) or structured form (period 2)	Medical errors near-miss, adverse events	1230 reports	27%	Not described
Kanter et al USA 2004 [14]	Retrospective 1 year	66146 premature	Discharge records	Medical error	1.2% premature neonates	Not described	Not described
Sharek et al USA 2006 [4]	Cross-sectional, retrospective	749 charts, 15 NICU	Trigger tool	Adverse event	0.74 AE per patient	40%	56%
Ligi et al France 2008 [3]	Prospective 8 months	388 neonates	Anonymous, non-punitive, voluntary	Iatrogenic event	25.6 IE‰ patient-days 0.69 IE per patient	29%	34%
Kugelman et al Israel 2008 [5]	Prospective Period 1: 3 months Period 2: 3 months	4 NICU 697 neonates	Anonymous, daily report by a iatrogenesis advocate Medical staff aware of the study in period 2	Iatrogenic event medical errors near miss event	20.2 IE ‰ patient-days 0.4 IE per patient	48.5%	83%
Snidjers et al Netherlands 2009 [15]	Prospective 12 months	8 NICU, 1 PICU 3859 neonates	Anonymous, voluntary, non-punitive	Critical incident	1.25 CI per admission	1.4%	Not described

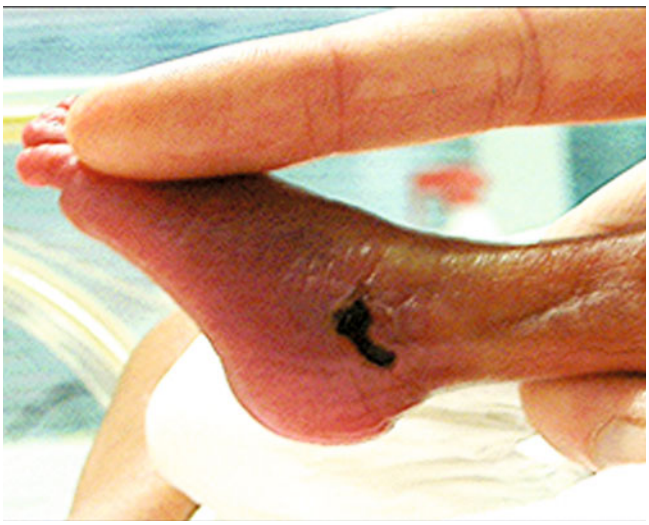

Fig. 26.1 Skin necrosis related to extravasation of fluids from peripheral venous catheter

As expected, the major risk factors of iatrogenesis are low birth weight and use of invasive procedures (central venous line and mechanical ventilation). Moreover, iatrogenesis is independently associated with an increased length of hospital stay [3-5].

26.3 Learning from Iatrogenic Events

Four steps are fundamental in developing risk management policy: identification, analysis, assessment and graduation, control of the risk.

26.3.1 Factors Contributing to Iatrogenic Event Occurrence

Multiple factors are invariably involved in the sequence of events that results in iatrogenesis occurrence. As described by James Reason in the "Swiss cheese" model of iatrogenic event occurrence, a set of circumstances coincide both in time and space to cause an event [9]. Human errors can contribute or cause events in an active or a latent way. Active failures comprise the unintentional lapses or slips (misinterpretation, error of attention or memory or selection) and the intentional mistakes (rule or knowledge based) or procedural violations. Active failures are immediate causes of an iatrogenic event. Latent failures are local and organizational conditions that facilitate event occurrence by providing a point of weakness. Factors include individual (training, health, awareness, fatigue) and team (communication, supervision) conditions, institutional (financial resources, safety culture) and environmental (staffing levels, equipment) factors, task conditions (workload, protocols) and patients attributes (age, language, personality, general health, social conditions).

26.3.2 Incident Reporting Systems

Incident reporting systems [19] refer to structured reporting, collation and analysis of iatrogenic events. Reporting systems are a key strategy for learning from errors. Mandatory reporting systems, on the basis of retrospective chart reviews, focus on errors associated with serious injuries or death. But errors resulting in serious harm are only part of the problem, and iatrogenesis is certainly underestimated by these systems. However, Sharek and colleagues recently described a trigger tool chart review that identifies efficiently adverse events in NICU [4]. By contrast, voluntary reporting systems focus on incidents that often result in slight injury, no harm or even those that are intercepted (so-called near-miss events). The intent is to identify and remedy vulnerabilities in systems to improve patient safety. Leape and colleagues emphasize the importance of a non-punitive, anonymous, and timely reporting system to effectively monitor adverse events. This approach has been shown to be effective in improving reporting [6, 20, 21]. Reporting can be facilitated by adding a trigger list of key incidents that reflect the nature of local clinical practice and areas of concern. Moreover, prospective methods of data collection demonstrate their superiority to cross sectional or retrospective methods [22]. Indeed, they seem more appropriate to identify preventable events, to assess the impact of error-prevention strategies, to study organizational and human factors and to assess the consequences of incidents. Furthermore, prospective incident reporting can be used as an educational process to convince clinical teams that errors contribute significantly to adverse events.

26.3.3 Investigation and Causation Analysis of Incidents

Investigations are needed for serious incidents or for recurrent ones. Debriefing (root causes analysis) is a key process to understand incident genesis and to learn from the incident to improve patient safety. The debriefing process has to be confidential, non-threatening, structured and timely. It is a collaborative process used to dissect events. The objectives are to determine what happened, to identify why it happened (underlying causative human and system factors) and to pinpoint lessons learned to make recommendations for improvement in a blame-free way. The lessons learned need to be fed back in a timely manner to the staff via meetings and/or a monthly bulletin.

26.3.4 Risk Assessment

Assessment and graduation of medical risk in terms of probability of occurrence or recurrence and harm potential is important for organizations in healthcare. Incidents can be reviewed retrospectively to rate the preventability and the severity. An alternative is to take a prospective approach in the technique of failure mode and effect analysis. The reliability and potential failures of any new system, process, equipment or service is systematically evaluated prior to exposure of patients and staff. Even if it is time-consuming, the proactive analysis facilitates assessment of potential risk prior to its implementation.

26.3.5 Limitations of Incident Reporting Systems

The prospective incident reporting systems are time-consuming in reporting and investigating incidents. Moreover, voluntary reporting can be inadvertently perceived as a means to blame and can paradoxically encourage defensive clinical practices. Another bias that is important to bear in mind is the underestimation of incidents by under-reporting or detecting.

26.3.6 Our Local Experience of Iatrogenic Event Reporting

Since 2005, we have used an anonymous, non-punitive and voluntary incident reporting system. The reporting form has been composed by a working group, which consists of neonatologists, one epidemiologist and referent nurses, on the basis of published work, professional expert devices and a pilot study in 2003. The form consists of three sections: administrative data (neonatal unit, patient identification, date of birth, discharge date, hospital length of stay or death), patient characteristics (birthweight, gestational age, sex, mechanical and/or continuous positive pressure support, central venous line) and narrative sections about incidents (date, age and weight, description, causative mechanisms). At the back of the form, a trigger list of 53 potential incidents was established to help reporting. Before the introduction of the form, several tutorial sessions and group discussions were held to familiarize nursing and medical staff with the incident report. The form was placed in the baby's chart from the time of admission until the discharge or death. Every month, two pediatricians independently review the reported incidents to confirm the diagnosis and to rate severity and preventability of each event. A monthly meeting is held by the working group to discuss rates and to enact preventive measures. A monthly bulletin composed of general data (number of admissions, activity level), of a summary of IE reports and nosocomial infections and of clinical lessons or guidelines is posted in units for feedback. Every 4 months, a staff meeting is organized with the nursing and medical team to discuss the rates and measures [3].

26.4 Strategies to Reduce the Risk: Components of Risk Management

Leadership and Safety Culture Leadership and safety culture (Fig. 26.2) are critical to convince staff that hazard and risk are not inevitable and can be managed to improve patient safety. Leaders must sensitize and encourage the entire staff in a collaborative and non-hierarchical process. The objectives are to convince that patient safety is a primary goal, that the system can be improved and the risk reduced. Leaders should emphasize the importance of blame-free incident monitoring as a positive means to improve quality, empower changes and allow development of targeted interventions. Leaders should also organize feedback, communication and promotion of patient safety.

Communication Communication with the parents is a major issue to help limit claims and complaints. Parent consent and information are key areas. Transparency is capital in the case of iatrogenic event occurrence. Communication between professionals is also essential to reduce risk and hazards.

Training, Induction and Competence As inexperience and unfamiliarity are key factors in error occurrence, continuous training is needed. Moreover, turnover is high in NICU and new staff are usual. All new staff must be aware of guidelines, policies and procedure.

Guidelines and Protocols Standards developed from evidence-based medicine are useful to assist professionals in decision in every steps of the care.

Audits Independent assessment of care and risk can be useful to implement preventive measures and to assess their effect during re-audit.

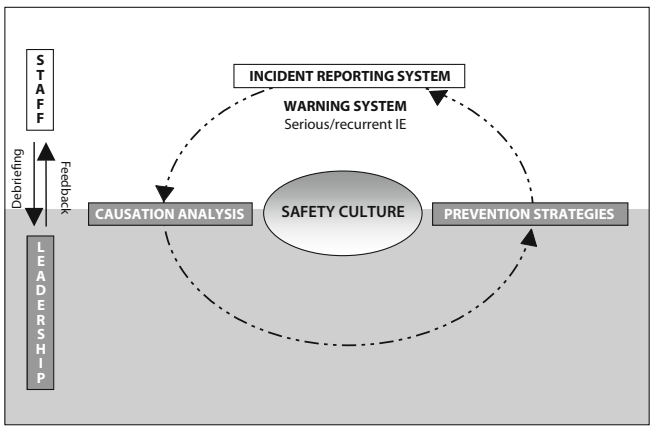

Fig. 26.2 Organization of risk management

Prevention of MEs: An Example of Risk Management MEs occur frequently in NICU with a real potential for harm. Prescription and administration, in particular smart pump programming, are two high-risk stages. Inexperience and intensity of workload are the major risk factor for occurrence. Even though medication management is a complex and multifaceted system, studies show that risk can be efficiently reduced and MEs prevented. First, the involvement of a NICU based pharmacist significantly reduces dosing errors [23]. Strategies to reduce error at the prescribing stage include: clinical staff education (indication documentation, formulation and dose regimen information), improving environment conditions at the time of prescription, implementation of standardized dosing references and perhaps use of computerized provider order entry (CPOE). CPOE could prevent 66% of MEs. However, CPOE may contribute to new types of errors and more studies are needed to assess the impact of such technology on MEs as CPOE systems are expensive [18]. Strategies to reduce errors at the administration stage include: use of standard concentrations for infusion, restriction of verbal orders to emergency situations, limitation of handwritten orders, requirement of double-checking at each step of the medication process especially for high-alert drugs (opiates, sedatives, insulin, anticoagulants, concentrated electrolyte solutions), storage of these drugs separate from ward stock, strict identification of syringes, handoff verification check. These measures have demonstrated their efficiency in reducing MEs.

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27

Guidelines and Protocols

Rinaldo Zanini

27.1 Introduction

Medicine nowadays offers many more services than ever. Progress in perinatal medicine through technology is a good example, although better living conditions for the population in general and for pregnant women in particular have also had a significant effect.

The complexities of modern medical procedures, joining different branches of medicine (Obstetrics and Neonatology to make Perinatology), and increased knowledge and available technology have contributed to today's good results. However, this has also imposed new behavioral and organizational models on physicians and healthcare workers to ensure that all these opportunities result in improved and consistent clinical outcomes for patients.

Organizational processes to promote team work are needed to facilitate the support and management of a healthcare process that is no longer in the hands of a single physician. The costs of health care have increased enormously because of advancing technology, the complexity of interventions and a continuing demand for better outcomes. These increased costs affect the healthcare systems of all industrialized countries and there is a risk that they will stunt medical progress in future years. Increased costs have tended to reduce the fairness of the healthcare system, making access less easy and decreasing its availability to users.

Because guidelines (GL) are systematic, repeatable, sharing and transparent, their use can contribute to a response to both control of costs and easy of access [1].

Complexity and cost are linked by the increasingly pressing need to provide high quality treatment with six fundamental attributes: safety, appropriateness, efficacy, efficiency, fairness and centrality for the user and his/her family.

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27.2 The Guidelines

A definition of GL has been agreed by the Institute of Medicine, USA:

> Guidelines are recommendations for clinical lines of conduct produced by way of a systematic process for the purpose of helping physicians and patients to decide on the most appropriate healthcare procedures in specific clinical circumstances [2].

Rather than being an imposition, GL are an aid to obtaining the most appropriate healthcare results, through clearly defined criteria of scientific validity (systematicity), which take into account the viewpoints of different stakeholders and which must therefore balance the interests of patients (clinical outcomes), physicians and healthcare providers (availability of resources).

The increasing use of GL has highlighted several problems, mainly related to the non-univocity of definitions and to not observing essential methodological prerequisites. The spread of low quality GL and GL that give contradictory recommendations are of growing concern [3–4].

GL require validity, which can be defined as the capacity to obtain an improvement in health. Validity in this sense is measurable. It is analogous to the efficacy of a drug, which can be evaluated for specific, determinable interventions, for example by way of randomized clinical trials, or of a diagnostic procedure, which can be characterized in terms of sensitivity, specificity, predictive value and probability ratio. The validity of the diffusion and implementation of a GL may then be evaluated in terms of the outcomes it produces [5].

Systematic reviews of these studies showed that in some contexts GL can change the behavior of healthcare practitioners and in some cases they can lead to significant improvements in clinical outcomes for patients [4–6]. These systematic reviews showed how a greater chance of obtaining positive results was associated with certain characteristics of GL. These include simplicity, local adaptability and the involvement of relevant professionals in their development.

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

An important problem highlighted by systematic reviews was the identification of methodological weakness of the available studies. This limited the ability to draw definitive conclusions about the validity of some treatments [6].

Assessment of the validity of a set of GL should be based on the positive changes its implementation induces on a specific pathological process. Such an assessment is made difficult by numerous confusing elements and by the complexity of the studies required. The concept of the validity of a GL has therefore shifted towards evaluation of adherence of a specific GL to the reference methodology, which is considered a guarantee of achieving the desired results.

GL may also be considered as tools for reducing the gap between current practice and alternative practices. They are therefore supporting tools to help make decisions for individual patients and for organizations by spelling out the benefits, risks and costs of different healthcare choices.

Based on these assumptions, the following methodological criteria have been identified:

1. The development of a GL and its evaluation must be oriented towards the results of the healthcare: the start of the process of forming (or using) a GL must always start from a precise healthcare context which is seen (or better still, evaluated) as problematic and therefore subject to improvement.
2. The GL must be based on the best evidence available.
3. The method used to summarize the available evidence must be rigorous, and follow a series of agreed steps: definition of the topic, formation of the writing group, definition of the method of bibliographical research and evaluation of the studies, dissemination and implementation.
4. The GL must contain an explicit classification of the strength of the recommendation: this is crucial and requires the need to give a "strength" to the various recommendations, based not only on the opinion of individual experts, but also on criteria arising directly from the quality of the studies which support the different recommendations of a GL.
5. The process of developing a GL must be multidisciplinary and involve users and patients. Experts in a discipline must not be the only ones to deal with a certain topic and others, who are in some way involved with the clinical management, including the patients, must also be included.
6. A GL must be adapted to the specific local circumstances.
7. A GL must take into consideration the available resources. This bridges the gap between experimental situations and the actual clinical context in which the individual practitioners work.
8. Once produced, GL must be disseminated and implemented in an appropriate manner. The simple act of publishing a set of GL in journals or congresses is not an effective way of promulgating their use in clinical practice.
9. The implementation of GL must be carefully monitored.
10. A GL must be regularly updated.

The main steps required for the formulation of GL are:

1. Formation of the work group.
2. An evaluation of the literature, aimed at establishing the best available research-based evidence for practice and researching GL already in existence.
3. Drafting the GL:
   - Systematic search of the literature;
   - Evaluation of the methodology of the studies;
   - Evaluation of the quality of the studies;
   - Production of a table of evidence, setting out the results of each study in terms of its conclusions and level of evidence. This step is the most difficult methodologically, as it assumes knowledge of epidemiology and the methodology of research; this step can be performed by persons outside the work group if there is nobody with the appropriate skills within it. The table of evidence should state the strength of evidence on which the GL is based, using a system such as that of the Scottish Intercollegiate Guidelines Network (SIGN) [7] (Table 27.1);

Table 27.1 Levels of evidence

1++	High quality meta-analysis. Systematic review of RCTs or RCTs with very low risk of bias
1+	Well conducted meta-analysis. Systematic review or RCTs with low risk of bias
1–	Meta-analyses. Systematic review or RCTs with risk of bias
2++	High quality systematic reviews of case-control or cohort studies High quality case-control or cohort studies with a low risk of confounding or bias and with a high probability that the relationship is causal
2+	Well conducted case-control or cohort studies with a low risk of confounding or bias and a moderate probability that the relationship is causal
2–	Case-control or cohort studies with a high risk of confounding or bias and a significant probability that the relationship is causal
3	Non-analytic studies (e.g., case reports, case series)
4	Expert opinions

Table 27.2 Strength of recommendations according to MERGE and SIGN criteria

A	At least one meta-analysis or a systematic review or an RCT classified as 1++ and directly applicable to the population, or a systematic review of RCT or a body of evidence consisting mainly of studies rated as 1+, directly applicable to the target population and with an overall consistency of results
B	A body of evidence consisting mainly of studies rated as 2++, directly applicable to the target population and with an overall consistency of results, or evidence extrapolated from studies rated as 1++ or l+
C	A body of evidence consisting mainly of studies evaluated as 2+, directly applicable to the target population and with an overall consistency of results, or evidence extrapolated from studies rated as 2++
D	Evidence level 3 or 4, or evidence extrapolated from studies rated as 2+

– Formulation of recommendations. On the basis of identified studies, recommendations for best practice can be made. These are then graded, according to the criteria listed in Table 27.2. This classification is drawn from the MERGE (Method for Evaluating Research and Guideline Evidence) [8] project and was adopted in 2000 by the SIGN. It partly changes a previous classification formulated by the AHCPR in 1993 [9] and has the advantage of incorporating quality aspects of the studies directly into the graded recommendations. It also gives appropriate weighting to randomized clinical trials, particularly for those issues (diagnosis, prognosis, etc.) for which other types of study (e.g., a cohort study), may be more appropriate.

4. Dissemination and implementation. These steps are an integral part of creating a set of GL. This section covers the identification of problems that may arise during the application of the GL and approaches for their resolution (e.g., incentivizing initiatives, structural and organizational changes, the use of specific tools such as reminders [paper or electronic] etc.) which can reach the site where the clinician actually makes the healthcare decisions [10].

By conducting an analysis of 431 sets of GL produced by international scientific societies between 1988 and 1998, evaluating them on the basis of essential criteria, such as their multidisciplinary applicability, completeness of the systematic review of the literature and gradation of the evidence, Grilli [3] demonstrated that 67% did not provide information

Table 27.3 Criteria for evaluating the validity of the guidelines' production process

	N/A	Yes	No
Responsibility and support for the development of the guidelines			
1. Do the guidelines identify the Institute/Organization responsible for the development and approval?	____	____	____
2. Do the guidelines give the source of finance and other support for the development?	____	____	____
3. If funds were received from commercial parties (e.g., pharmaceutical industries), do the guidelines give explicit details on the nature and level of the client?	____	____	____
4. Have any potential conflicts of interest of the parties providing finance or support been taken into consideration in a satisfactory way?	____	____	____
Composition of the guideline development group			
5. Is there a description of the individuals (professionals, interest groups including the patients) involved in the development of the guidelines?	____	____	____
6. Have all those involved made a formal declaration of interest?	____	____	____
7. Does the group contain representatives of all key disciplines?	____	____	____
8. Does the group include patients or their representatives?	____	____	____
9. Have each individual participant's potential conflicts of interest been taken into consideration in a satisfactory way?	____	____	____
Evidence identification and summary			
10. Is there a description of the methods used to collect the evidence on which the recommendations are based?	____	____	____
11. Are the methods of collecting the scientific evidence adequate?	____	____	____
12. Are the sources of information used to develop the guidelines adequately referenced?	____	____	____
13. Is there a description of the methods used to obtain the opinion of possible interested parties not included in the group developing the guidelines?	____	____	____
Assessing the strength of the scientific evidence			
14. Is there a description of the methods used to assess the strength of the evidence?	____	____	____
15. Do the guidelines make explicit links between the recommendations and the strength of the supporting evidence?	____	____	____
16. Overall, are the methods used to rate or weigh up the scientific evidence satisfactory?	____	____	____
Pre-test and external evaluation			
17. Have the guidelines been submitted to an Independent external review by experts or by an external panel?	____	____	____
18. Have the guidelines been pilot tested?	____	____	____
19. If the guidelines were pilot tested, has explicit information been given on the methods adopted and the results?	____	____	____
Review			
20. Do the guidelines provide explicit details on how they will be subjected to review and updating?	____	____	____
21. Do the guidelines identify the parties responsible for the review and updating?	____	____	____
22. Do the guidelines give a date scheduled for the review or an expiry date?	____	____	____
Global assessment of the validity of the guideline development process			
23. In the guidelines, is there an accurate summary of the methods, content and recommendations?	____	____	____
24. Overall, have the problems of potential bias and conflicts of interest been adequately dealt with?	____	____	____

Table 27.4 Criteria for the assessment of the validity of the guidelines' content and format

	N/A	Yes	No
Clinical applicability/flexibility			
1. Is there a description of the patient population(s) to which the guidelines are considered to be applicable?			
2. Is there a description of the professional groups for which the guidelines are considered to be applicable?			
3. Is there a description of the circumstances (clinical and non-clinical) in which exceptions can be made in the use of the guidelines?			
4. Is there a description of the ethical issues likely to arise in the using the guidelines?			
5. Is there an explicit description of how the patients' preferences must be taken into consideration in the application of the guidelines?			
6. Overall, do the recommendations cover all the significant clinical circumstances (including the diagnostic processes, clinical treatment and consultations)?			
7. Are the guideline recommendations consistent with each other?			
Clarity			
8. Are the main topics presented clearly?			
9. Do the guidelines describe the conditions to be diagnosed, treated or prevented in unambiguous terms?			
10. Do the guidelines describe in unambiguous terms the various options for the decisions on the condition?			
11. Do the guidelines identify current unacceptable or ineffective practices?			
12. Are the recommendations written in unambiguous terms?			
13. Can the main recommendations be found easily?			
Description of the likely costs and benefits			
14. Is there an adequate description of the health benefits that are likely to be gained from the application of the clinical recommendations?			
15. Is there a description of the potential harms or risks that may occur as a result of specific clinical recommendations?			
16. Is there an adequate description of the costs and expenditure likely to be incurred for specific recommended clinical lines of conduct?			
17. Have the potential costs and benefits been estimated appropriately?			
18. Are the issues regarding administrative/managerial competence clearly identified?			
19. Are the recommendations supported by the estimated benefits and possible harms and risks of the intervention (with the relevant strength of the evidence)?			
Development of local healthcare protocol, dissemination and implementation			
20. Do the guidelines describe recommended methods for the development of local healthcare protocols?			
21. Do the guidelines identify which professional groups should be involved in the development of local healthcare protocols?			
22. Do the guidelines identify key elements to be considered in local healthcare protocols?			
23. Do the guidelines identify key elements for which information should be provided to patients?			
24. Are there examples of reminders for individual patients?			
Clinical Audit			
25. Do the guidelines identify specific clinical outcome indicators for the areas covered by the guidelines?			
26. Are core targets and standards identified?			
27. Do the guidelines define the core data for reporting the relevant clinical care results?			
28. Does the core data for clinical care include key indicators of a) risk b) severity?			

on the staff involved, 88% did not provide information on the bibliographic search strategies, and 82% did not grade the evidence. Only 5% of GL reviewed satisfied all the criteria. However, there was an improvement with time, especially in respect of the search strategy (from 2% to 18%) and grading of the evidence (from 6% to 27%). Similar studies revealed the same problems, meaning that tracking down good quality GL cannot be taken for granted.

If it is possible to find GL with good validity, what strategies can be used to identify valid GL? It might be possible to adopt GL that have already produced positive results in terms of health outcomes. However, such experiences are relatively scarce and cannot be reproduced in different contexts. Account needs to be taken therefore of the local situation, ensuring the relevance of GL, which may lead to recommendations that are different to the originals.

An alternative strategy is to adopt stringent, validated methodological criteria to assess the available GL, or to construct evaluation "grids", which can be used by clinicians and by the final users. A recent systematic review analyzed available studies on this topic. It came to the conclusion that more than one of the tools had characteristics of such validity as to be of practical use, the one developed within the context of the European project AGREE [11], being one of the most widespread and useful. This evaluation tool focuses attention on the methodological and formal aspects described above, structuring the analysis and the evaluation of them in a format that is easily understood and usable (Tables. 27.3 and 27.4). In this way it is possible to evaluate a set of GL, adhere to a good development process and therefore, by inference, achieve the desired health effect. Thus, the tool is based on theoretical assumptions (the best available at the time) rather than on empirical evidence, which it is hoped can be accumulated with a wider diffusion of this type of evaluation.

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28

Physical Environment: the Thermal Environment

Gunnar Sedin

28.1 Overview

During intrauterine life, heat production by the fetus results in a fetal temperature that is about 0.5°C higher than the maternal temperature [1]. After birth, the newborn infant is exposed to air and surfaces, which have a much lower temperature than that previously experienced in utero. The skin surface at birth is covered with amniotic fluid, causing heat loss through evaporation in an environment with a low vapor pressure [2]. As a result the body temperature of the infant is lowered, and the rate of this reduction is influenced by the temperature of the environmental air in the delivery room and the delivery of its flow. This gives rise to thermogenic responses that increase basal heat production [1, 3] and the skin circulation may decrease to lower the heat losses [4].

Heat balance in newborn infants depends on the heat transfer between the infant and the environment [5–8]. This transfer is related to the temperature and humidity of the environmental air (the vapor pressure), the flow velocity of that air (which causes a sense of cooling of the skin) and the temperatures of the surfaces facing the infant (ceiling, walls of room or incubator or bedding material) and the temperature of the surfaces in direct contact with the infant [5, 6, 8, 9].

After birth, the immediate interventions required to avoid body cooling are to carefully wipe off the amniotic fluid from the skin surface with cotton fabrics to lower the loss of heat through evaporation, and to cover the infant with a warm, dry towel or blanket, or both, to lessen the exposure of the infant's skin to the environment [2, 6]. The infant born at term or moderately preterm can be covered with a blanket and then placed on the mother's chest [10–12], but extremely preterm infants usually need other types of measures to maintain their body temperature, usually placement in an incubator or under a radiant heater [5, 6, 13–16] and, if necessary, mechanical ventilation with warm and humidified gas [7, 17–19].

28.2 Routes of Heat Exchange

Heat exchange between the infant and its environment occurs through the skin and through the respiratory tract. It has previously been difficult to estimate the heat exchange between the infant and the environment. The introduction of new techniques to measure the evaporation rate from the skin [6, 14, 20, 21] and from the respiratory tract [7, 21, 22] has permitted the heat loss through evaporation to be determined [6]. It is also possible to calculate the heat loss through other modes of heat exchange, such as radiation, convection (air movements), and conduction (in direct contact with a piece of material or skin) and to determine the heat loss per unit surface area and from the total body surface area [8], and also to take into consideration the proportions of surface area of the body participating in the different modes of heat exchange [5, 6]. In addition, the modes of heat exchange between the infant's respiratory tract and the environment can be determined.

28.3 Water and Heat Exchange Between the Infant's Body Surface and the Environment

Heat exchange through evaporation, radiation, convection and conduction can be calculated with knowledge of the transepidermal water loss (loss of water through the skin), the temperature of the infant's skin (T_{skin}), the temperature of the walls facing the infant (T_{wall}), the temperature of the ambient air (T_{amb}), the temperature of the material on which the infant is placed (T_{bed}), and characteristics of the material in the infant's environment, using the equations given in the following [2, 5].

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

28.3.1 Determination of Water Loss from the Skin

In the absence of forced convection (air movements), and if the effect of thermal diffusion is disregarded, the process of water exchange through a stationary water-permeable surface can be expressed in terms of the vapor pressure gradient immediately adjacent to the surface [20].

The evaporation rate (ER; g/m^2 h) from the infant's skin can thus be determined by a method based on calculation of the water vapor pressure in the layer of air immediately adjacent to the vapor pressure and the distance from the evaporating surface [20]. In this zone, the relationship between the vapor pressure and the distance from the evaporating surface is linear [20]. If the gradient in this layer is known, the amount of water evaporated per unit time (transepidermal water loss, TEWL; g/m^2h, which is a mean value of cutaneous water loss) can be calculated [20] according to the following equation:

$$TEWL = 0.92 \times ER_{(a,b,c)} + 1.37$$

where $ER_{(a,b,c)}$ is the arithmetic mean of ER measured from the chest, an interscapular area and a buttock [8, 9, 21, 22].

The gradient method (Evaporimeter, Servomed AB, 511 21 Varberg, Sweden), allows quick measurements of free evaporation without disturbing the infant [20, 22].

28.3.2 Calculations of Heat Exchange Between the Infant and the Environment

28.3.2.1 Exchange at the Surface

Heat exchange through evaporation (H_{evap}), can be calculated if TEWL is known [8, 9, 20–22] according to the equation:

$$H_{evap} = k_1 \times TEWL\ (3.6 \times 103)^{-1} \qquad (W/m^2)$$

where k_1 is the latent heat of evaporation (2.4×10^3 J/g), and 3.6×10^3 is the correction factor for time (s).

Heat exchange through radiation (H_{rad}) can be determined if the mean temperature of the skin (T_1; K) and the mean temperature of the surrounding walls (T_2; K) are known:

$$H_{rad} = S_0 \times e_1 \times e_2 \times (T_1^4 - T_2^4) \qquad (W/m^2)$$

where S_0 is Stefan-Boltzman's constant (5.7×10^{-8} W/m^2 K^4), e_1 is the emissivity of the skin, and e_2 is the emissivity of the surrounding walls (0.97).

Heat exchange through convection (H_{conv}) can be calculated if the mean temperature of the skin (T_1; K) and the mean temperature of the ambient air (T_3; K) are known:

$$H_{conv} = K_2\ (T_1 - T_3) \qquad (W/m^2)$$

where K_2 is the convection coefficient (2.7 W/m^2 K). The coefficient for convection given above and used in many studies referred to in this chapter has usually been determined in measurements on adult human skin [23]. A convection coefficient suggested as being more valid for newborn infants [24] can alternatively be used and Hconv will then be 48 % higher.

Heat exchange through conduction (H_{cond}) can be determined if the temperature of the skin (T_{skin}; K) and the temperature of the bed (T_{bed}; K) are known:

$$H_{cond} = k_0\ (T_{skin} - T_{bed}) \qquad (W/m^2)$$

In this equation k_0 is a conductive heat transfer coefficient. With the thermal conductivity characteristics of most regular mattresses the heat loss through conduction is very low.

The extent of heat exchange between the body surface area and the environment thus depends on the type of heat exchange, on the position and geometry of the body [6, 8] and on the magnitude and frequency of body movements. As different modes of heat exchange are unequally influenced by changes in the body position, the relative contribution of different modes of heat exchange might vary with time. Heat exchange is often presented per unit area of body surface exposed to the ambient air or facing the walls of the incubator.

28.3.2.2 Heat Exchange Between the Infant's Respiratory Tract and the Environment

The expired air is usually more humid, than the inspired air, and has a higher water vapor pressure. This implies that evaporative loss of water and heat takes place from the respiratory tract. Convective heat transfer, usually of low degree, also occurs in the respiratory tract. In research, these two processes are considered together [25]. In the newborn, heat can also be gained through the respiratory tract if the infant inspires very warm air with a high humidity. Because of the alternating displacement of air during the respiratory cycle, convective and evaporative heat transfer in the respiratory tract is complex. When ambient air, which is cooler than the body, passes along the mucosa of the infant during inspiration, it gains heat by convection and gains water vapor by evaporation from the mucosa. On reaching the alveoli, the air is at thermal equilibrium with the central body temperature and is saturated with water. During expiration, the expired air may become a little cooler than the body temperature before it leaves the infant [7, 21].

28.3.2.3 Determination of Water Loss from the Airway

Respiratory water loss (RWL) is usually included in measurements of total insensible water loss when these are performed

with ventilated chambers, but it can be estimated separately [26, 27]. Hey and Katz [26] and Sulyok and colleagues [27] found that the respiratory water loss was higher at a low ambient humidity than at a high humidity. In other studies, data on respiratory water loss, oxygen consumption and carbon dioxide production were provided [7, 17, 18, 21, 28–32].

28.3.2.4 Calculation of Heat Exchange Between the Infant's Respiratory Tract and the Environment

The exchange of heat through convection in the respiratory tract, H_{conv-r}, is calculated from the air volume ventilated per unit time (V = ventilation volume) and from the temperature difference between expired and inspired air ($T_E - T_I$) according to the following relationship:

$$H_{conv-r} = V \times r \times c\ (T_E - T_I)\ m^{-1} \qquad (W/kg)$$

where V is ventilation volume per unit time, r is density (1 g = 0.880 L), c is the specific heat (1 J × g^{-1} × $°C^{-1}$), m is the body weight (kg) [8].

Because of the alternating inspiratory warming and expiratory cooling of the air, the convective heat exchange in the respiratory tract depends mainly on the temperature of the inspired air. In human infants nursed in incubators there is a very small difference between the temperatures of the inspired and of the expired air, and convective losses are small.

Evaporative heat exchange from the airway (H_{evap-r}) depends on the difference in water content between the expired and the inspired air. This is the respiratory water loss [17, 19, 33]. As the formation of water vapor in the respiratory tract requires thermal energy, the amount of heat exchange by evaporation per unit time is expressed by the following equation:

$$H_{evap-r} = k_1 \times RWL\ (3.6 \times 10^3)^{-1} \qquad (W/kg)$$

where k_1 is the latent heat of evaporation of water (2.4×10^3 J/g), RWL is the respiratory water loss (mg/kg min) and $(3.6 \times 10^3)^{-1}$ is the correction factor for time.

28.4 Heat Exchange in Incubators, under Radiant Heaters and in Heated Beds

When it was realized that a good thermal environment increases the chances of survival of newborn infants, attempts were made to provide them with a warm environment. The ambient temperature influences the survival rate and oxygen consumption in newborn infants [3, 26, 33] and a body temperature of around 37°C is appropriate for newborn infants at rest [31]. At a high ambient temperature full-term infants start to sweat, and if they are fed cold glucose through a gastric tube, sweating is inhibited [34].

28.4.1 Incubators

In a convectively heated incubator, the warm air supplied should be directed so that both the air temperature of the incubator and the incubator walls are kept warm. If the air flow velocity is lower than 0.1 m/s, the convective heat transfer will depend on the gradient between the skin and the air temperature, and the vapor pressure gradient close to the skin surface will be maintained, avoiding an increased evaporative rate due to increased air flow velocity [8, 23, 24].

An evaluation of environmental and climate control in incubators has shown that the air flow velocities and capacity for humidification vary markedly between different types of incubators. Also, the wall temperatures vary considerably between different incubators [32] and it is therefore necessary to determine the air flow velocities, humidity and wall temperatures carefully before calculating heat exchange. With air flows higher than 0.2 m/s, there will be a forced convection.

28.4.2 Radiant Heaters

A radiant warmer placed over an open bed platform provides good accessibility and visibility for the care of the newborn infant and has therefore become widely used in neonatal intensive care.

Infants nursed under a radiant heater gain heat, but they may also have extensive heat losses through evaporation and convection, and also, from some surfaces, through radiation [14, 15, 35–38], making it difficult to estimate the relative contributions of different modes of heat exchange. Because of the free movements of air above the infant's body surface, both evaporative and convective heat loss may increase as a result of a high air velocity.

28.4.3 Heated Beds

In the 1990s, Sarman and co-workers found that infants weighing 1000 g or more can be kept warm by placing them on a heated water-filled mattress [39] early after birth. By covering most parts of the body except the face, direct heat exchange between the infant's skin and the environment through other modes can be almost eliminated in a large proportion of the body surface.

Preterm and term infants can maintain a normal body temperature during skin-to-skin care, if they are placed lying naked except for a diaper on the mother's or father's chest, and are covered with the mother's clothing or a blanket [10–12].

Only parts of the head are exposed to the environmental air [10–12] and will exchange heat with the environment.

28.5 Water and Heat Exchange Between the Skin and the Environment

In a series of studies, water evaporation from the skin surface of infants nursed in incubators has been determined by use of the gradient method (the ServoMed Evaporimeter), and transepidermal water loss (TEWL) [13, 21, 22, 40] and heat exchange have been calculated [2, 5, 6]. In these studies the body temperature has been maintained at 36-37°C, except when the effect of warming has been investigated, and the ambient humidity has been kept at 50% except when the effects of different humidities have been analyzed. Evaporative heat loss is usually insensible, but might become sensible when term infants are nursed in a warm environment and start to sweat [34]. Some infants born at term and nursed in a warm environment do not begin to sweat [34], and if they do, the sweating is preceded by an increase in skin blood flow. In preterm infants the sweat glands are non-functional [26].

28.5.1 Heat Exchange During the First Hours after Birth

Immediately after birth, when the infant's skin is covered with amniotic fluid, there is an enormous evaporative heat loss from the skin surface. This evaporative heat loss decreases gradually during the first hours, whether the infant is nursed in the delivery room or in an incubator [2], (Fig. 28.1, top two diagrams). Heat loss through radiation is high if the infant's skin is facing the walls of the delivery room and is much lower if it is facing the inner walls of the incubator [2] (Fig. 28.1, middle diagrams). The heat loss through convection is lower than that through radiation but is much higher when the infant is nursed in room air (Fig. 28.1, lower left diagram) than when nursed in the incubator (Fig. 28.1 lower, right diagram).

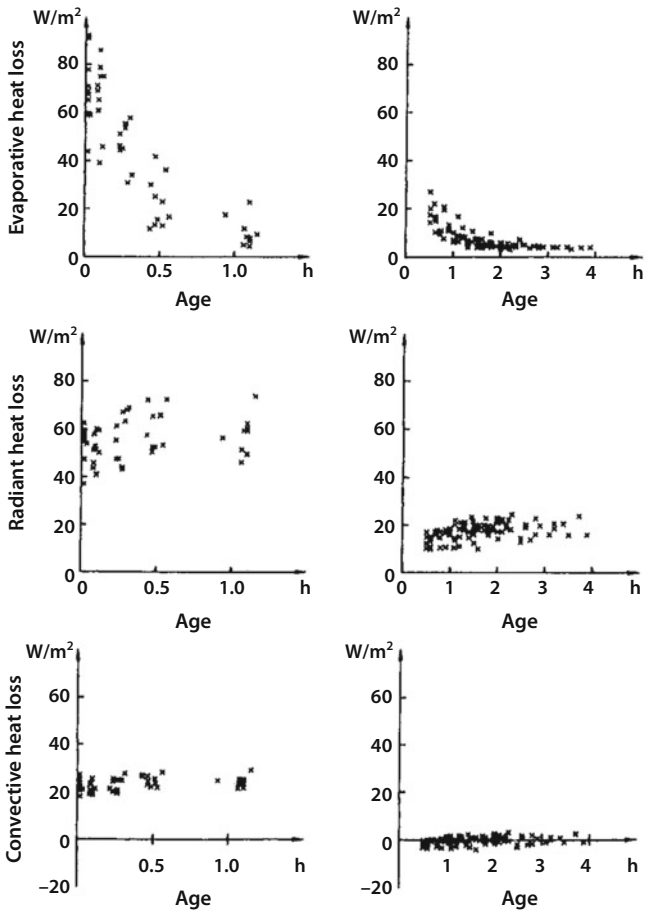

Fig. 28.1 Heat exchange between the skin of full-term infants and the environment immediately after birth and during the first 1 to 4 hours postnatally. The infants were not washed or wiped. Data presented in the left-hand scatter diagrams are based on measurements in infants initially placed at the delivery bed or at the mother's chest and later in a cot. After the measurements made 1 and 5 minutes after birth, the infants were covered with a towel between the measurements. Data in the right-hand diagrams are based on measurements in infants who were covered with a towel after birth and until they were placed in an incubator [2]

28.5.2 Transepidermal Water Loss During the First 4 Postnatal Weeks

The transepidermal water loss from the skin surface of the newborn infant depends primarily on the gestational age (GA) at birth [13], but it is also influenced by the postnatal age [40]. The transepidermal water loss in newborn infants is also lower in small for gestational age (SGA) [21] than in appropriate for gestational age (AGA) [13, 21, 40] infants. There is an exponential relationship between TEWL and gestational age in AGA infants when measurements are made during the first day after birth [13]. This relationship prevails over the first 4 weeks after birth, even though the difference in TEWL between the most preterm and the full-term AGA infants gradually diminishes with increasing age. The evaporation rate and TEWL both depend on the ambient relative humidity or ambient vapor pressure [13, 21]. This relationship seems to be valid for all gestational ages and is also valid for all postnatal ages studied [13, 21, 40, 41].

28.5.3 Heat Exchange During the First Day after Birth

The evaporative heat exchange is directly proportional to the amount of water that evaporates from the infant's skin, and shows the same type of relationship to GA as TEWL [5, 21]. When infants are nursed in an ambient humidity of 50%, the evaporative heat exchange may reach 50 W/m^2 of the body surface area in the most preterm infants [5], whereas in term

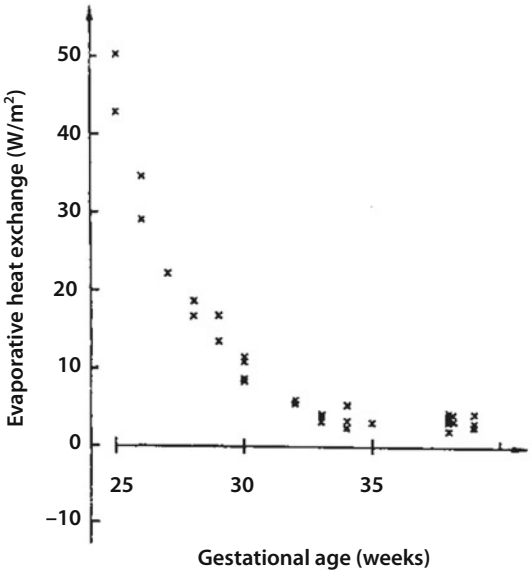

Fig. 28.2 Heat exchange through evaporation at ambient humidity of 50% in relationship to gestational age. Measurements were made during the first 24 hours after birth in preterm infants and during the first 30 hours in term infants [5]

infants it is close to 5 W/m^2 (Fig. 28.2). The high evaporative heat loss in very preterm infants makes it necessary to have a very high ambient temperature in the incubator to maintain a normal body temperature of 36.0°-37.0°C in these infants.

The servocontrol system of the incubator that controls the skin temperature, regulates the temperature of the air, which also leads to changes in the temperature of the incubator walls and thereby influences the heat exchange through convection and radiation.

The most preterm infants can even gain heat through these modes of heat exchange. Preterm infants were nursed in an incubator with a relative humidity of 50%, at a higher ambient vapor pressure than the more mature infants need [21, 40]. This means that the heat exchange between the very preterm infants and their environment could have been even greater, if comparisons had been made at equal ambient vapor pressures instead of at equal ambient humidities.

In studies of the effects of different ambient humidities, it was found that infants born at a GA of less than 28 weeks need a T_{amb} of around 40°C to maintain a normal body temperature at an ambient humidity of 20%, whereas full-term infants need a T_{amb} of around 34°C at this ambient humidity (Fig. 28.3). Nursing very preterm infants at a higher ambient humidity means that a lower ambient temperature can be sufficient to maintain a normal body temperature [5].

28.5.4 Heat Exchange at Different Ambient Humidities

Evaporative heat exchange between the skin of very preterm infants and the environment is highest at a low humidity [5],

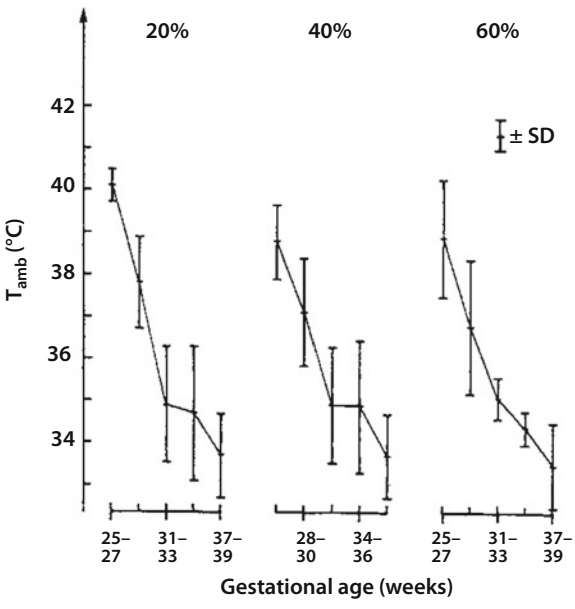

Fig. 28.3 The relationship between gestational age and the ambient temperature needed to maintain a normal body temperature at three different humidities of 20%, 40% and 60%. Measurements were made during the first 24 hours after birth in preterm infants and during the first 30 hours in term infants [5]

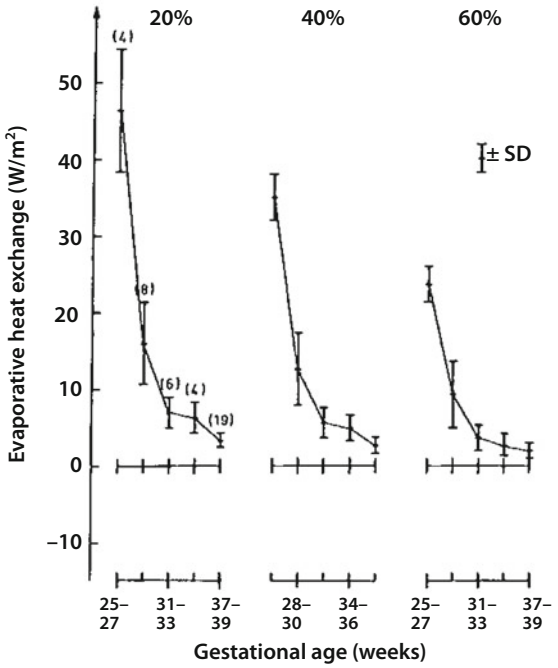

Fig. 28.4 Heat exchange through evaporation in relation to gestational age at relative ambient humidities of 20%, 40% and 60%. *SD*, standard deviation [5]

and lower at higher ambient humidity. In fact, heat exchange through evaporation in the most preterm infants is twice as high at 20% as at 60% ambient humidity. Other modes of heat exchange are all influenced by the ambient humidity (Fig. 28.4). The sum of the different modes of heat exchange, in the different GA groups is almost the same, but the total heat loss cannot be calculated in this way, as the proportions of the body surface area exchanging heat in different ways are not known.

28.5.5 Heat Exchange During the First Weeks after Birth

The high evaporative losses of heat from the infant's skin that are seen in the most preterm infants during the first days after birth will gradually decrease with increasing postnatal age [6]. Heat loss through radiation is low early after birth in the most preterm infants born at 25–27 weeks of gestation. Heat loss from radiation will, however, be the most important mode of heat exchange after the first postnatal week. In infants born at a GA of 28 or more weeks, the radiative heat exchange is the most important mode of heat exchange from birth and it gradually increases with age. The heat exchange through convection is low in infants nursed in incubators. Initially there is a gain of heat through convection in the most preterm infants early after birth.

During the first weeks after birth the relative magnitude of the different modes of heat exchange markedly depend on the ambient humidity (Fig. 28.5). In infants born at 25–27 weeks of gestation and nursed in a dry environment, evaporative heat exchange will be the most important mode of heat exchange for more than 10 days after birth. At an ambient humidity of 60% this mode of exchange is the highest only during the first 5 days. Thereafter, the radiative heat exchange is the most important mode of heat loss [6].

The total heat exchange between the infant's skin and the environment can be calculated if the body surface area of the infant and the fractions of this area that participate in the different modes of heat exchange are known [6, 21, 26]. This is very difficult with available methods, especially if estimations are to be made over a longer period of time [6]. The total heat exchange is basically dependent on the metabolic rate.

28.5.6 Heat Exchange Between the Infant's Skin and the Environment During Care under Radiant Heaters

Several authors have considered that a lower vapor pressure in the ambient air [42] and more convective air currents under the radiant heater [35] cause an increase in evaporative water loss and heat exchange. More recent determination of the evaporation rate from the skin of term, moderately preterm and very preterm infants nursed in incubators with 50% ambient humidity and under a radiant heater, have shown that the evaporation rate from the skin surface of full-term infants is significantly higher under a radiant heater than in an incubator [14]. In moderately preterm infants born at 30 to 34 weeks of gestation, the evaporation rate from the skin is significantly higher at an environmental humidity of 30% than at 50%, and in very preterm infants with a GA of less than 30 weeks, the evaporation rate is higher under a radiant heater than in an incubator at an environmental humidity of 50%.

A transparent heat shield positioned over the infant can lower the heat exchange, (occurring through both convection and evaporation) between the infant and its environment, decrease convective air currents and increase humidification

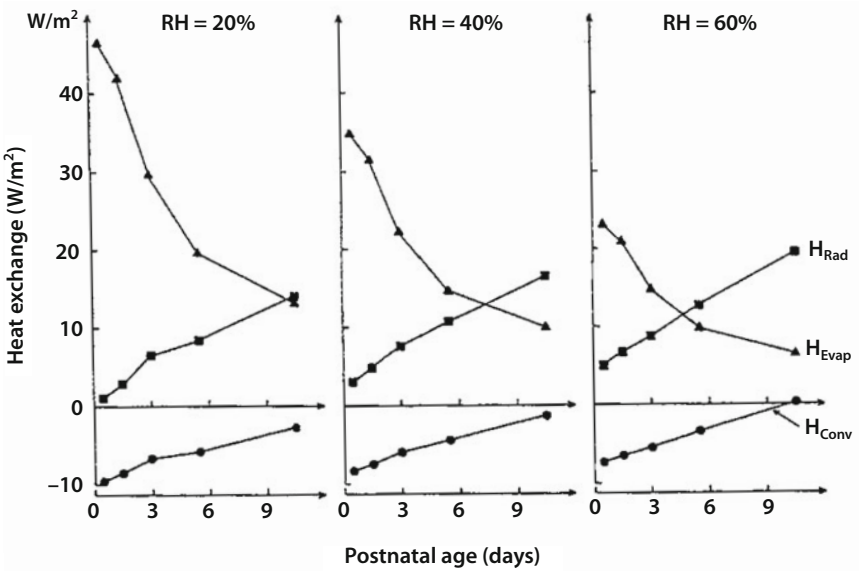

Fig. 28.5 Heat exchange between the infant and the environment in relation to postnatal age at relative ambient humidities (RH) of 20%, 40% and 60% in infants born at 25 to 27 weeks of gestation [6]

[35] and diminish the water loss [42–44]. It has been suggested that a bed platform that can be used with a radiant heater or with a hood might make it easier to adapt to the mode of care that such an infant needs [45].

28.5.7 Ambient Humidity Influence on the Rate of Skin Barrier Maturation in Extremely Preterm Infants

In infants born at 24–25 completed weeks of gestation, and nursed in incubators at 50% ambient humidity, water loss was higher than that previously found in infants born at 25-27 weeks of gestation [46].

To investigate if the level of radiant heaters (RH) in which the preterm infants are nursed, might influence their postnatal skin maturation, TEWL was determined in twenty preterm infants (GA 23–27 weeks) nursed at postnatal ages (PNA) of 0, 3, 7, 14 and 28 days [47]. The results indicate that the level of RH influences skin barrier development, with more rapid barrier formation in infants nursed at a lower RH. The findings have an impact for promoting skin barrier integrity in extremely preterm infants (Fig. 28.6) [47]. The individual decrease in $TEWL_{50}$ was consistently more pronounced in the RH_{50} group than in the RH_{75} group. There were no differences in body temperature, skin temperature, or ambient temperatures (T_{amb} and T_{wall}) between the 2 study groups. The total fluid intake and serum sodium levels during the period 0–14 days of PNA are presented in Fig. 28.6.

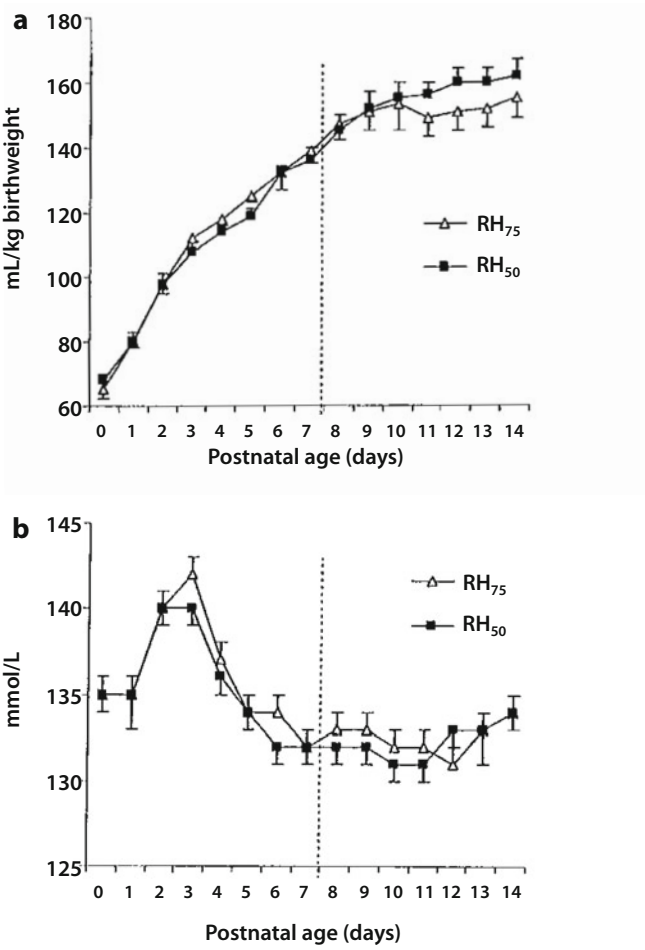

Fig. 28.6 Daily total fluid intake (**a**) and serum sodium levels (**b**) at postnatal ages (PNA) of 0 to 14 days in infants nursed at RH_{75} or RH_{50} from PNA 7 days (*indicated by dotted lines*). There are no statistically significant differences between study groups [46]

Table 28.1 Effect of activity level on insensible water loss and oxygen consumption

Activity level	IWL_S	IWL_R	IWL_T	VO_2
0	6	6	12	8
1		8		9
2		9		10
3		10		11
4		12		12
5	7	16	23	13

Insensible water loss from the skin (IWL_S) and from the respiratory tract (IWL_R) and their sum (IWL_T) in g/kg/24 h, and oxygen consumption (VO_2; L/kg/24 h), in full-term appropriate for gestational age infants at different levels of activity. From [29].

Table 28.1 further documents that there are no significant differences between the group of infants that were nursed at a RH of 75% and those nursed at a RH of 50% (Table 28.1).

28.5.8 Heat Exchange Between the Infant's Skin and the Environment During Phototherapy

Phototherapy has been reported to increase insensible water loss in newborn infants [35, 42]. This loss of water and heat might be caused by altered barrier properties in the skin or by increased respiratory water loss. However, other studies have indicated that in thermally stable term and preterm infants [36] there is no increase in the evaporation rate from the skin surface during phototherapy.

28.5.9 Heat Exchange Between the Infant's Skin and the Environment During Care on a Heated Bed

An infant on a heated bed gains heat through conduction. Some losses of water and heat from the airway will take place, depending on the vapor pressure of the room air. Sarman and colleagues showed that infants weighting ≤1000 g

could be kept warm when nursed on a heated water–filled mattress [39]. In a 2 week trial with infants whose birthweights were between 1300 and 1500 g, Gray and Flenady found that that weight gain was lower and body temperature higher in those who were cot-nursed with warming than in those who were nursed in an incubator [48].

28.5.10 Heat Exchange Between the Infant's Skin and the Environment During Skin-to-Skin Care

After the introduction of skin-to-skin care for newborn infants [12], Bauer and colleagues showed that preterm infants less than 1 week of age and with a birthweight of less then 1500 g were not exposed to cold stress during 60 minutes skin-to-skin care in stable preterm infants [10]. Similarly, preterm infants born at 28 to 30 weeks of gestation and studied during the first and second week after birth increased their body temperature during 1 hour of skin-to-skin contact with no significant change in oxygen consumption [11]. More immature infants, born at 25 to 27 weeks of gestation, showed no increase in oxygen consumption but a decrease in rectal temperature during the same duration of skin-to skin contact during the first week after birth.

During the second postnatal week, the body temperature did not change in infants born at 25 to 27 weeks of gestation during skin-to-skin care [11]. It has been suggested that infants born at 25 to 27 weeks of gestation might have a very high evaporative heat loss during the first week after birth, causing cold stress [6, 13].

28.5.11 Keeping the Very Preterm Infant Warm Early after Birth: the Effect of Thin Clothing

Newborn infants are at risk of losing heat soon after birth. It is therefore of great importance to wipe off amniotic fluid from the skin surface, remove wet linen, and to wrap the infant in pre-warmed blankets or alternatively, placing the infant skin to skin on the mothers chest to use her body as a heat source [12].

When an infant is placed in an environment where the temperature and humidity is low, the evaporative heat and water exchange will be high and air movements in the environment may further increase the loss of heat through evaporation, conduction, convection and radiation. It is therefore of outmost importance to keep the infant warm. In a laboratory setting we present data on the evaporation rate from a semi-permeable membrane positioned above a membrane [2, 5, 13, 49].

28.6 Water and Heat Exchange Between the Respiratory Tract and Environment

Water and heat exchange from the respiratory tract takes place with each expiration. Using indirect methods, it has been shown that respiratory water loss depends on the humidity of the inspired air, with lower losses occurring at a high humidity [26, 38]. The respiratory water loss (mg/kg min) can be determined using a flow-through system with a mass spectrometer for measurements of gas concentrations [7–9, 17, 18, 28–30, 32]. In an environment with an ambient humidity of 50% the evaporative heat loss from the airway will be moderate [28], i.e., in term AGA infants the insensible water loss from the respiratory tract (IWL_R) and that from the skin (IWL_S) will be of equal magnitude [28]. The evaporative heat loss from the respiratory tract ($H_{evap–r}$) and the evaporative heat loss (H_{evap-s}) from the skin will also be of equal magnitude under these conditions.

The evaporative loss of water and heat from the airway depends on the ambient humidity both in lambs [7] and infants [28], with lower losses at a high than at a low humidity. Whereas IWL_S decreased from 9 to 2g/kg/24h in term infants when the ambient humidity was increased from 20 to 80%, the corresponding change in IWL_R was much smaller, i.e., from 9 to 5g/kg/24h [28]. In infants placed in a calorimeter, Solyok et al [27] found that the respiratory heat loss was between 0.07 and 0.22 W/kg, or 3-10% of the total heat production from metabolism and about 40% of the insensible heat loss. In term infants, RW_L and H_{evap-r} may increase during activity by up to 140% of the values at rest [29].

In Table 28.2, data are presented on IWL_S and IWL_R. During moderate heat stress both lambs and infants can increase their RWL and H_{evap-r} without increasing their oxygen consumption and CO_2 production [17, 30]. Exposure to radiant heat can alter the respiratory water loss in a lamb while leaving the oxygen consumption and CO_2 production unchanged. RWL is directly proportional to the rate of breathing [17, 30, 31], which means that lambs and infants will lose more water and heat when they have a high rate of breathing (Fig. 28.7). The oxygen consumption will increase if the heat stress induces bodily movements with higher levels of activity [29].

28.6.1 Respiratory Water and Evaporative Heat Exchange before and after Intubation

In non-intubated lambs, exposed to a radiant heat source, RWL increased from 10.5 to 33.4 mg/kg/min, while the respiratory rate increased from 54 to 161 breaths/min and the oxygen consumption and CO_2 production were unaltered [21]. During exposure to the same heat source, intubated lambs increased their respiratory water loss from 8.1 to 18.7

Table 28.2 Effects of phototherapy on respiratory values in neonates

	n	RWL (mg/kg/min)	VO_2 (mL/kg/min)	VCO_2 (mL/kg/min)	RR (breaths/min)
Before	11	4.4 ± 0.7	5.9 ± 0.9	4.0 ± 0.7	48 ± 7
12 min	11	4.4 ± 0.6	5.9 ± 1.0	3.9 ± 0.6	
24 min	10	4.1 ± 0.8	5.5 ± 1.1	3.8 ± 0.7	
36 min	9	4.2 ± 1.0	5.4 ± 1.0	3.6 ± 0.6	
48 min	11	4.4 ± 0.7	5.9 ± 1.1	3.9 ± 0.7	51 ± 11
60 min	9	4.6 ± 0.9	5.9 ± 1.1	4.1 ± 0.8	
After	9	4.8 ± 0.8	6.1 ± 0.9	4.1 ± 0.4	

Respiratory water loss (RWL), oxygen consumption (VO_2), carbon dioxide production (VCO_2; mean ± SD) and respiratory rate (RR) in full-term infants before, during, and after 60 min of phototherapy. From [22].

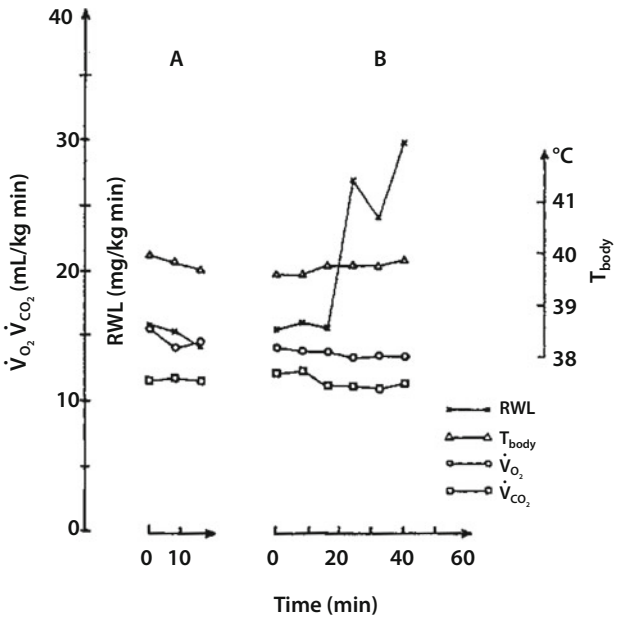

Fig. 28.7 Respiratory water loss (RWL), body temperature (T_{body}), oxygen consumption (VO_2) and CO_2 production (VCO_2) in a 6-day-old lamb before (A) and during (B) heat stress. From [30]

mg/kg/min and their rate of breathing from 46 to 125 breaths/min. In intubated lambs both the oxygen consumption and CO_2 production increased significantly [21].

28.6.2 Respiratory Water and Evaporative Heat Loss in Relation to GA

RWL was found to be highest in the most preterm infants and lower in more mature infants; they were usually asleep during the measurements [18]. Studies were performed in incubators with 50% ambient relative humidity and an ambient temperature that allowed the infant to maintain a normal and stable body temperature. RWL per breath (mg/kg) was almost the same at all gestational ages and the higher RWL values found in the most preterm infants as compared to the more mature ones was thus due to the higher rate of breathing [46].

28.6.3 Respiratory Water and Evaporative Heat Exchange During Phototherapy

When term and preterm infants [22] were exposed to phototherapy in the absence of heat stress, there were no significant changes in RWL, oxygen consumption, CO_2 production or rate of breathing [22] (Table 28.2).

28.6.4 Respiratory Water and Heat Exchange During Mechanical Ventilation

In clinical neonatal care with a warm and humid environment or with warm and humidified gas supplied from a respirator, heat exchange through evaporation and convection between the respiratory tract and the environment is low [7, 27, 28]. Infants who inspire cold air with a low water vapor pressure will have high evaporative loss from the respiratory tract and will also lose heat through convection. These losses may become clinically significant, for instance during transport in a cold climate [19].

The concept of an optimum thermal environment for newborn infants evolved during the 1960s [50]. This idealized setting, called the neutral thermal environment, is characterized as the range of environmental temperature within which the metabolic rate is at a minimum and within which temperature regulation is achieved by non-evaporative physical processes alone. There is no reason to believe that the normal temperature of infants is different from that of adults. An infant's internal temperature within the range of 36.5° to 37.5 °C (with a diurnal variation of ±0.5°C) is probably normal. Because body temperature is a measure of only the balance between heat production and net heat loss, a normal body

temperature should not be confused with a "normal" metabolic rate [50].

A cold-stressed infant depends primarily on mechanisms that cause chemical thermogenesis. When an infant is stimulated by cold, norepinephrine [51] is released, inducing lipolysis in brown fat stores principally found in the interscapular, paraspinal and perirenal areas. Because of the protein thermogenin, brown fat can decouple oxidative phosphorylation and break down fats to produce heat, without the inhibitory feedback loop of adenosine triphosphate (ATP) production. Triglycerides in the fat are broken down to fatty acids and glycerol. The fatty acids enter thermogenic metabolic paths that end in the common pool of metabolic acids. Brown fat is turned on in response to skin thermoreceptors prior to a decrease in core temperature, and shivering is initiated by a decrease in core temperature.

Glycolysis may also be stimulated during severe stress when epinephrine released from the adrenals activates glycogen stores, which may result in transient hyperglycemia [52]. Lowered blood sugars in cold-stressed infants also have been reported [53], possibly caused by inhibition of glycolysis, by lipolysis, or by exhaustion of glycogen stores.

28.7 Summary

The exchange of heat between the infant's skin and the environment is influenced by the insulation provided by the skin, the permeability of the skin, and environmental factors such as the ambient temperature and humidity, air-flow velocity, and the temperature and characteristics of the incubator surfaces facing the infant. Evaporative heat loss from the skin is the major component of heat exchange in the most preterm infants early after birth, and these infants gain heat through convection and, in a very dry environment, possibly also through radiation when they are nursed in an incubator. As water loss from the skin surface of the most preterm infants decreases with postnatal age, heat loss through evaporation from the skin also decreases. Their need for a high ambient temperature also diminishes, and with the lower temperature of the incubator walls, the heat loss through radiation then increases and the heat gain by convection is changed to a low loss of heat during the first weeks after birth.

Infants nursed under a radiant heater gain heat through radiation and, as a result of the low ambient humidity, evaporative loss of water and heat may be high in very preterm infants. The loss of heat through convection greatly depends on how the infant is protected from high air velocities by arrangements made under the radiant heater, and also on the magnitude of movements in the nursery.

In term infants nursed in incubators with an ambient humidity of 50%, the evaporative water and heat loss from the respiratory tract and from the skin are about the same magnitude. Respiratory water and evaporative heat loss is thus low in term infants. The respiratory evaporative water loss and heat loss per breath are of about the same magnitude in calm preterm and term infants. In preterm infants, the evaporative heat losses from the skin are much more important than such heat losses from the respiratory tract. The evaporative heat loss from the airway might be considered important only in infants with high motor activity or with tachypnea, especially if they are nursed in a very dry environment. Mechanical ventilation with dry and cold air also results in high loss of heat from the respiratory tract through evaporation and convection.

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29

Information and Psychosocial Intervention in Neonatology

Massimo Agosti

29.1 Introduction

Maternity is a unique experience calling for shared responsibility in protecting the baby, the parents and their environment. Mood disorders (stress, depression) can occur and sometimes interfere with the complex network of relationships that lead to satisfactory family and infant relationships.

Women with antenatal psychosocial risk factors are more likely to have postnatal depression [1]. Various investigations have supported routine antenatal and postnatal screening for psychological mood disorders. An assessment screening questionnaire developed by Matthey and colleagues was used to collect information on the frequency of psychosocial risk factors in over 2000 pregnant women in Australia, as well as the impact on clinical caseloads. The most common risk factors were personality and life stresses (24%), followed by psychiatric history (20%), current mood (12%), childhood abuse (9%), family violence (5%: interestingly, more women reported being violent toward their partner than their partner being violent toward them); the least frequent risk factor was lack of support (3%) [2].

Even hospitalization of the premature infant in the neonatal intensive care unit (NICU) is considered as a "psychological crisis" in the family, causing feelings of powerlessness and stress, especially for mothers.

29.2 Preterm Birth and Family Stress

In Italy approximately 6000 very low birth weight (VLBW, less than 1500 g) births occur annually. Survival rates for small preterm infants have improved, especially those of VLBW. Although the birth of an infant with VLBW poses a considerable challenge to parents, there is little information about how it affects family stress. For an event like preterm birth, different sources of parental stress have been identified. One of the most important is early separation of the mother and infant, which may adversely affect attachment between the mother-child dyad [3]. Consequently, feelings of anxiety and depression are frequent in mothers of preterm infants and appear to be more intense during the hospitalization of the child in the neonatal intensive care unit. Maternal depression has been associated with impairment of child cognitive, emotional, and behavioral development. Early maternal depression or other psychological symptoms may have varying effects on preterm infants. The relationship of maternal mental health on parenting behavior is important for preterm populations because maternal psychological status and other social factors (e.g., socioeconomic status, maternal substance abuse, multiple birth, or other life stressors) have been found to correlate with childhood emotional and mental well-being in children of school age, who were of low-birth-weight or preterm. Signs of parental distress can emerge during hospitalization, which is why staff should adopt a holistic approach to understanding the uniqueness of each family and provide appropriate support.

If a problem is diagnosed during pregnancy, parents should be forewarned. If this does not occur, most women and their partners will not give serious consideration to the possibility of preterm delivery or illness in their newborn baby and admission to a neonatal unit. Parents are unfamiliar with the potentially complex problems their infant faces and there is uncertainty about the future. A lack of understanding and uncertainty are major sources of stress. In addition, maternal health is often compromised at this time. Mother and baby are separated when the infant is admitted to the neonatal unit, and this may last many months. Although in some hospitals a visit to the neonatal unit is a routine part of antenatal care, the neonatal unit is generally an alien environment for most parents. Units are often noisy, bright, and hot. They can be overcrowded and parts will be "high tech." Parents rarely know the neonatal unit staff before their baby is admitted, and the language and behaviors they encounter can contribute to

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an overwhelming feeling of isolation. The sickest preterm infants may be in hospital for many months, and visiting can be difficult, exhausting, and a financial drain for parents, especially as neonatal services become more centralized. All these factors put strain on the parents' relationship: a relationship is more likely to breakdown during the months after preterm delivery. Some couples, however, feel the experience makes their relationship closer, at least initially. Generally, stress and anxiety are higher in mothers than in fathers, and lessen with the passage of time. For some parents, the stress experienced is similar to that seen in adults diagnosed with post-traumatic stress disorder. High levels of stress may last beyond the first year of their infant's life, and the level and duration of stress may not be directly related to how preterm or how sick their baby was. In addition to high levels of stress and anxiety, these parents are more prone to clinical depression, which may be difficult to recognize.

Responses and feelings similar to those of a classic grief reaction can be encountered: shock, denial, anger, guilt, acceptance, and adjustment. Several models have explored how parents cope with having their baby in the neonatal unit. Various mechanisms are seen, however, and a single is not appropriate for all parents. Some coping strategies include trying to gain a deeper understanding of the problems, establishing a degree of control over the situation, seeking social support from other people, and escaping from or minimizing the apparent severity of the situation. These mechanisms are used to varying degrees by individual parents. There is a systematic difference between mothers and fathers. Mothers tend to look for support from others and to search for an explanation for what has happened, whereas fathers are more likely to try to minimize the situation, often by concentrating on supporting their partner.

29.3 Interventions

A better understanding of the sources of stress and how parents try to cope allows appropriate interventions for the family. A complex network of structural, practical, social and psychological interventions can promote acceptance by mothers and fathers of this challenging event. When designing neonatal units, great emphasis is placed on effective layout, lighting, and noise reduction. Facilities for families to stay close to their baby are usually provided, and rooms for parents allow mothers and fathers to relax and meet other parents. Play areas for siblings have been incorporated into some units. This more "family-orientated" approach to care is helped by less restrictive visiting policies in neonatal units. Most units allow parents and siblings open access if they comply with local infection control measures. Thus a more sensitive approach could affect faster bonding between mother and sick newborn (i.e., 24/24 open access to NICU, kangaroo mother-care, promotion of maternal/human milk).

Prenatal counseling is an aspect on which many units are currently engaged, especially if a prenatal diagnosis has been made. Members of the neonatal team meet with parents before the birth to discuss a likely admission and the approach that will be taken for the infant's problems. Parents are encouraged to visit the unit before their baby is born to familiarize themselves with the environment and the staff. After delivery, it is good practice to discuss medical and nursing issues in detail with parents to inform and, when possible, to involve them in decision making from an early stage. Parents should be informed as soon as possible about investigation results and any change in clinical condition. After overcoming their first moments of confusion and fear, parents can also help with the care of their preterm baby. This may extend beyond simple but important measures, including "skin-to-skin" contact, providing skilled care such as tube feeding, toileting practices, and joining intensive "developmental care" programs. Parents of other preterm babies can give support through "buddy" programs or informally [4]. Counseling through external organizations or formal support can be helpful even for families whose babies are not critically ill. This can involve various professionals, such as psychologists, social services, religious advisers, ethics committees. Written information about the neonatal unit, describing specific conditions or procedures may be useful. Routine contact between the neonatal unit and social services may allow financial support to be provided for the parents [5].

Despite intensive treatment and full medical support, patients in a neonatal unit may die. Although there are substantial differences between countries and cultures, a decision to limit active treatment may be considered because of the inevitability of death or a prognosis that indicates a very poor quality of life. Death, however it comes about, is a desperate time for affected families. In some cases, parents want to be involved in decision making at these times. They need full and frank information, given in a compassionate manner by experienced staff who know the family and their baby. In most cases, the decision to stop or limit treatment is made with senior medical and nursing staff. Family, friends, and external bodies (such as religious leaders and support groups) do not often have a substantial role in the decision to withdraw treatment but contribute to family support afterwards. Mothers and fathers may differ in the way they grieve and cope with their loss. Mementoes, formal contact with senior staff during the weeks after the death, and contact with a bereavement support worker or group may all help the process. Most families begin to come to terms with the death during the first year — not forgetting the child but adjusting to life without him or her. Bereaved parents often need factual information that may help explain why their baby has died. Without autopsy, important information can be missed, and in many neonatal units, postmortem examination is always considered and offered to the parents if appropriate. High profile cases where there have been procedural inadequacies and anxieties about organ retention have contributed to a decrease in the number of autopsies carried

out. Additionally, there has been a natural reluctance by parents to authorize further "suffering" for their infant and a lack of awareness of the questions that remain unanswered [6].

Discharge home, although an exciting time for families, can also be a time of extreme anxiety, and so a formal approach to "discharge planning" is often adopted. Mothers "room in" with their baby to promote bonding, establish feeding, and learn practical skills that might be needed. Support for the family in the community once the infant is discharged can also be arranged, including specialist neonatal nurses, primary care health staff (for example, health visitors, general practitioners), social workers, and national or local family support groups. Although managing the immediate stress of discharge home is important, it should be recognized that although practical issues may become easier with the passage of time, for some families considerable levels of stress and anxiety remain long after the discharge itself. Psychological support should be an integral part of neonatal follow-up programs.

Parents and families of babies who are admitted to a neonatal unit are exposed to a variety of stressors, and may face extremely difficult decisions in unique and unfamiliar situations. Many studies suggest that current VLBW follow-up programs might benefit from the addition of psychological and family services to traditional neurodevelopmental assessment programs, particularly during the neonatal period and up to infant age of 2 years. 10% of mothers of infants with VLBW reported severe symptoms of psychological distress during the neonatal period, five times the rate for term mothers, while almost one third of mothers of infants with VLBW had clinically significant levels of depression and anxiety. For mothers of high-risk infants with VLBW, significant symptoms recurred when the child was 2 years old. The neonatal period affords an opportunity to identify mothers who are most at risk using standardized, simple screening techniques [7]. Such identification and referral for treatment may prevent the development of more severe symptoms, which can interfere with effective parenting while the provision of social support may also effectively buffer distress [8]. By targeting this support appropriately, staff on neonatal units can provide a more complete package of care [9].

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30

Neonatology and the Law

Vittorio Fineschi and Emanuela Turillazzi

30.1 Introduction

Neonatology is an area of medicine that has received much attention in recent years. This interest primarily concerns the constant progress in this field and the inherent ethical and medico legal problems. The extensive debate about some issues reflects both the technical and scientific progress that has been made in neonatology. Tightly argued issues such as medical malpractice litigation have reached crisis proportions in the neonatology and pediatrics. Moreover pediatric claims are very expensive because damages cover the life of the child and the juries tend to be very sympathetic toward children and their families [1]. Care of extremely preterm infants at the limits of viability and the "brain damaged newborn" are some of the more contentious areas of legal medicine in neonatology.

Neonatologists' concerns about a potential complaint, inquiry or lawsuit influence their practice of medicine in potentially positive ways such as developing audit procedures, maintaining better communication with parents and families, ensuring timely documentation of procedures, communication of complications, but also negatively such as increased prescribing of drugs, referrals and diagnostic testing, and avoiding treatment of certain conditions.

Since recognizing "red flags" can lessen the chances of malpractice claims against neonatologists [2], this chapter focuses on some issues that are highly vulnerable to claims of medical negligence and on high-risk situations. The principles are primarily the same for any doctor working in any country under any clinical circumstances. However, subtle differences in the law will apply to individual cases depending on circumstances and the jurisdiction that applies [3].

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30.2 Neonatal Resuscitation Practices

30.2.1 Presuppositions

In recent years a lively debate has developed concerning the decision to interrupt or not to initiate resuscitation procedures on low gestational age newborns [4–6]. In many countries all over the world, rulings by multidisciplinary study groups is evidence of the importance of this debate, with neonatologists, pediatricians, obstetricians and bioethicists working together to develop a framework for making decisions about whether or not to initiate resuscitation of "at risk" newborns. At the same time there have been requests for juridical regulation of neonatal resuscitation practices as well as for clarification of the role of parents in decisions in this area.

The practical approach to the resuscitation of extremely low birth weight (ELBW) infants varies greatly between countries and, in the same country, between different centers, reflecting a paucity of evidence and consequent uncertainty among clinicians. Different possibilities for intervention in the care of ELBW infants reflect both the need to ensure that mother and newborn are offered adequate assistance and for them to be spared useless, painful and ineffective therapies.

The central issue regards the "of uncertain vitality" infants born between 22 and 25 weeks of gestational age whose treatment at birth is one of the most challenging for neonatologists [7].

When we are confronted with extremely low gestational age (22 weeks' gestational age (154–160 days' intrauterine life), there is general agreement that decisions regarding the treatment of the mother must be based on her health, that cesarean section must be performed only when indicated by the mother's clinical condition and that the newborn should be provided with care aimed at promoting comfort. On the other hand, with increasing gestational age if the newborn shows capacity to survive, the newborn's condition must be carefully assessed at birth and resuscitation performed if appropriate.

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The presence of favorable objective clinical criteria (presence of attempts at respiration, valid cardiac frequency, recovery of skin color) suggest proceeding with extraordinary therapies, and intensive treatment of the newborn can becomes more appropriate.

It is widely believed that when gestational age is at 25 weeks, cesarean section may be performed for fetal reasons, the newborn must be resuscitated and subjected to intensive, extraordinary therapies, except those cases who present with severely compromised clinical conditions suggesting the impossibility of survival.

In this scenario, and after years of discussion across this troubled terrain, some crucial issues are intensely debate and remain to be solved.

30.2.2 Critical Assumptions

First, consider the assumption that all medical decisions about the non-institution or withdrawal of intensive care should be based on gestational age. There is actually a fair margin of error in the estimation of gestational age, so that in doubtful cases clinical evaluation of the newborn is of major importance. Neonatologists should take particular account of the newborn's conditions at birth, obstetric history and response to resuscitation. Recent studies [8] based on data from pregnancies induced with *in vitro* fertilization programs (the only case in which dating can be absolutely sure), show that the best echographic dating of pregnancy may show an error of between –10 and +7 days. The timing of the menstrual cycle is even more inaccurate with deviations of as much as 14 days [9]. There are obvious effects of such a range of error on decisions in a grey area where even a few days may lead to non-institution of neonatal resuscitation or its institution. Since neonatal prognosis is conditioned by many independent predictive factors (birth weight, use of steroids before birth, multiplicity of pregnancy) [10], it is evident that the chronological criterion of gestational age alone might lead to a dangerous simplification in evaluating decisional paths, giving excessive value to one single parameter. It is well known that the prognosis for very preterm children varies with the place of birth (level 3 perinatal center or not), the attitude of obstetricians and pediatricians about the interventions used, gestational age, postnatal age, and later co-morbidities [10]. Nevertheless, many authors still consider gestational age, although imperfect, the best indicator of the infant maturation (i.e. survival capacity) and guidelines refer to gestational age to guide behaviors and clinical choices [11, 12].

The second assumption is that one of the unique features of neonatal bioethics is its focus on guidelines that specify which extremely preterm babies should not receive resuscitation [13]. No other area of medicine has been as focused on such policies or as specific in delineating treatment limitations. In other areas, guidelines are broad and general, with much room for individual clinical judgment and professional discretion. Some authors have reported that policies for newborns appear very different from those for other patient populations [9]. In fact, even in critical situations burdened by high mortality or morbidity (for example adult patients with cardiac arrest after trauma, cardiac arrest in children following severe trauma, adult patients with a primary hemorrhagic stroke), a low chance of survival with possible long-term significant and disabling sequelae does not result in any recommendation not to resuscitate [14–16].

Finally, a point of fundamental importance is the central position of parents in the decision-making process relating to therapies administered to their premature newborns. Since resuscitation requires immediate decisions and prompt and timely actions, parents should be provided with understandable and detailed information about the complications of prematurity and associated life expectancy and its quality. Full psychological support should be provided. In the event of conflict between the parents' requests and the physician's decision, a joint solution should be sought, taking into consideration the best interests of the fetus and newborn.

30.2.3 The Crux of the Matter

The crux of the matter is whether strict guidelines, reference standards based on the gestational age parameter, and authority rules are necessary.

In a decisional area burdened by such limited prognostic certainties, an individual approach is infinitely more acceptable than a statistical approach: any decision ought to be based on the individual circumstances of each newborn rather than on reference to guidelines, especially if these are based on gestational age [17]. Physicians do not need rigid rules based on inflexible gestational age and birth weight guidelines but guidance to address the difficult and trying issues associated with infants born at the margins of viability and the ability to make a realistic assessment of the infant's clinical condition [18].

The principles regarding treatment of low or very low gestational age newborns should be the same of those followed for other patients, there is no need for specific policy statements. Generally, the purpose of guidelines is to improve knowledge regarding neonatal outcomes, to provide consistency in periviability counseling, and to promote informed, supportive, responsible choices. Flexible guidelines are well accepted and can be used by all neonatologists, obstetricians, and nurses who provide care to pregnant women and infants at extremely early gestational ages. However, resuscitation decisions for extremely preterm infants should be approached in the same way as for other patients. They should be individualized by objective and individual prognostication, which is as accurate as possible and which takes into account all relevant clinical characteristics.

30.3 The "Brain Damaged" Newborn

30.3.1 Presuppositions

New insights into the origins of cerebral palsy have recently transformed the old concept that most cases of cerebral palsy begin in labor. Many factors can damage the "developing" fetal brain including prepartum, intrapartum and postpartum ischemia/hypoxemia, developmental abnormalities, genetic factors, metabolic disease, infections, autoimmune and coagulation disorders, and maternal and fetal drug use [19]. Clinical signs of brain damage are often considered to result from intrapartum asphyxia and to be a consequence of perinatal obstetric and/or neonatologic mismanagement, leading to litigation. Contrary to previous beliefs and assumptions, clinical epidemiological studies indicate that in most cases the events leading to cerebral palsy occur in the fetus before the onset of labor, or in the newborn after delivery. It has been suggested that 70–80% of cases of cerebral palsy (CP) are due to prenatal factors and that birth asphyxia plays a relatively minor role. Intrapartum asphyxia is believed to account for around 10% of CP in term and near-term infants [20]. Notwithstanding advances in our understanding into the causation of cerebral palsy, defining a causal relation between acute intrapartum events and cerebral palsy remains very difficult.

Since the occurrence of a brain damaged newborn frequently leads to allegations of peripartum mismanagement, understanding the time of onset of the brain lesion is of paramount importance to medical and legal professionals. In Courts much of the debate focuses on whether or not there is evidence of acute intrapartum hypoxia and if so, whether the care provided was timely and of a satisfactory standard [21, 22].

30.3.2 The Timing of Perinatal Hypoxia

The timing of perinatal hypoxia is complex and incompletely understood. It is traditionally based on clinical, laboratory and physiological data, which are nonspecific markers of a difficult birth [23, 24]. These non-specific intra-partum markers provide poor information about the timing and duration of an asphyxiating insult [25]. A detailed clinical history, autopsy, placental and cord examination, laboratory tests and genetic studies may explain the cause of death and the time of onset of the neuropathology. Brain histological examination can provide useful information about the timing of an hypoxic-ischemic lesion: the patterns of perinatal brain injury depend on the etiology and the stage of development of the fetal nervous system, since the vulnerabilities of gray and white matter depend on post-conceptional age and on neuro-anatomic site [26, 27]. New insights into the mechanisms of neonatal hypoxic-ischemic brain injury have effectively challenged the old concept that most cases are the results of an acute hypoxia during labor and delivery.

In October 1999, the International Cerebral Palsy Task Force published a consensus statement for defining a causal relationship between acute intra-partum events and cerebral palsy with the aim of defining "an objective template of evidence to better identify cases of cerebral palsy where the neuropathology began or became established around labor and birth". It established three "essential criteria", which should be met before attributing cerebral palsy to an intra-partum hypoxic event and a number of "criteria that together suggest an intra-partum timing, but are non-specific" [21].

Laboratory studies may be useful for defining the timing of an hypoxic event, i.e., acid-base disturbance assessed by means of umbilical-artery pH measurement, serum concentration of brain-specific creatine kinase isoenzyme BB (CK-BB), other serum factors (blood lactate, hypoxanthine, aspartate-aminotransferase, erythropoietin beta-endorphin), factors meas- ured in the cerebrospinal fluid (lactate, neuron-specific enolase, lactate dehydrogenase, hydroxybutyrate dehydrogenase, fibrinogen degradation products, ascorbic acid). Imaging studies such as head ultrasonography may show typical injury patterns (periventricular leukomalacia, lesions of the basal ganglia, cerebral edema, ischemic infarcts). Cranial computed tomography (CT) may demonstrate ischemic or hemorrhagic lesions, generalized or focal edema, diffuse cerebral atrophy with ex vacuo ventricular dilatation due to severe hypoxemic insult. On CT, periventricular leukomalacia can be visualized as a region of decreased attenuation, occasionally intermixed with areas of increased attenuation due to secondary hemorrhage. However, magnetic resonance is the imaging modality of choice in the assessment of cerebral hypoxic lesion.

In cases of neonatal death it is essential that the contribution of post mortem examination is complemented by toxicological, microbiological and genetic investigations [28]. The histological study of the brain with traditional histochemical techniques can provide relevant data since it is well known that the distribution and the histological pattern of lesions in the brain changes dramatically depending on mechanism, severity and timing of the insult. There are numerous studies to define histological, histochemical and immunohistochemical markers to provide increasingly accurate information about the time of onset of hypoxic-ischemic brain damage. However, there are also fragmentary and contradictory data in the literature, citing experimental studies performed only on animals. Many reports are of experimental studies that have used markers with late expression (>24 hours) and which are therefore of limited use for the detection of cases of perinatal hypoxic-ischemic brain damage due to intra-partum asphyxia [27]. These neurobiological insights into cellular responses implicated in perinatal brain damages, and the characterization of the various mechanisms involved, might open new avenues for understanding the time of onset of cerebral hypoxic-ischemic lesions and for effective therapeutic strategies [29]. Immunohistochemical techniques in

studies on animals and humans have identified reliable and reproducible brain tissue markers of hypoxic-ischemic damage. The immunohistochemical picture obtained using a panel of selected antibodies (chaperonins HSPs, ORP-150, COX2) have identified a chronology of expression of the different markers of hypoxic-ischemic brain damage in newborns. This has been correlated with the duration of the insult and ascribed to the stimulation of the different cell types and to a different response by the same cells to the ischemic insult. Some immunohistochemical markers seem to be more reliable than others in the evaluation of the timing of the neonatal hypoxic-ischemic damage [30]. The results of histologic and immunohistochemical investigations suggest that the differential diagnosis of neonatal hypoxic –ischemia may be better defined in terms of the precise timing of the injury [29].

30.4 Informed Consent

Inadequate informed consent is a very frequent basis for alleged medical malpractice, particularly in neonatology where there are unique issues because consent is not obtained from the patient but from the family often during a very emotionally stressful period. Inadequate communication (between the obstetrician and pediatrician/neonatologist, the nurse and physician, or the healthcare team and the family) often leads to allegations of malpractice [1].

The relationship between the physician and patient's parents and families is critically determined by the quality and the amount of information and the use of language. There has to be differentiation between simply reporting a situation and imparting information. The two dimensions are not equivalent: the provision of news (i.e. reporting) and the provision of information are not the same thing. A fundamental issue is that of truth and of the amount of information to be provided to the newborns' family. How and how much is to be told about the newborn's disease, outcome and prognosis? Which are the risks of certain treatments? Which predictable sequelae should be revealed to the newborn's parents?

The purpose of information and communication is to enable parents to make informed choices about the health care of their baby. However, a crucial issue is the distinction of the rights of the newborn and the capacity to make autonomous choices. Great care is necessary when dealing with this issue; attention must be focused on what is to be done with a baby who has not the capacity to make autonomous choices. The issue of informed consent has become more difficult because of new technologies, which have increased the range of available interventions and the possibilities of diagnosis and cure. These have also meant that such issues are more likely to find themselves debated in a judicial setting.

When dealing with the issue of informed consent in the neonatology and pediatrics, there are some unique challenges that are different to those in adult medicine.

A crucial question that has major ethical importance is that of parents' rights in the decision making process. There are several aspects related to the newborn's parents, i.e. the recipients of information, that need to be taken into account by the physician: their desire for knowledge; their desire to participate in the therapeutic choices; their psychological state; their level of knowledge. There is a distinct risk that imparting information becomes only an episode of learned, specialist discourse which is unintelligible to parents. There is therefore a special burden on the neonatologist to ensure that the newborn or infant's parents understand the explanations and information given.

Since malpractice claims are filed by parents whose newborn babies have died or have suffered poor outcomes, the value of informed consent in reducing malpractice losses may be undermined by the tendency to approach informed consent as a means to "protect" the physician rather than to communicate with parents and to share with them clinical decisions, which are often very challenging.

Open communication with parents and families should represent the gold standard of the physician-parent-patient relationship. The physician and the parents have specific competences: those of the physician are technical and scientific; those of the parents are human, relating to pain, and perception of the quality of life. Thus cooperation is needed to achieve the aim of optimal health for the newborn baby. Parents should be active participants in a dialogue that is important to the shared decision-making process; the physician has a duty to ensure that comprehension is achieved and that the parents themselves are acting in the best interests of their child.

30.5 Conclusions

The unfortunate reality of the current practice of medicine is that physicians will likely be faced with a malpractice claim at some point in their career. Neonatologists and pediatricians are not immune. Recent publications addressing the malpractice situation as it relates to pediatrics [31] showed that pediatricians accounted for 2.97% of claims, making it 10th among the 28 specialties in terms of the number of closed claims. Pediatrics ranks 16th in terms of indemnity payments (28.13%) [32].

It is now believed that few of the medical malpractice claims filed actually involve negligence on the part of the physicians. A study performed as part of the Harvard Medical Practice Study concluded that less than 2% of the injuries in a hospital setting that are caused by negligence ever result in a malpractice complaint [33]. In neonatology and pediatrics failure to diagnose was the leading reason for child-related payments (18%), followed by sub-standard performance (9%), delay in diagnosis (9%), and poor management (6%) [34]. The most prevalent causes of malpractice suits against pediatricians involve errors in diagnosis. Part of the reason

for this lies in the nature of pediatrics: obtaining a history from a child is very different from that of an adult. Additionally, the pediatrician often tries to provide medical care and advice over the telephone, without the advantage of having the patient in front of them [35].

A patient's decision to sue his or her physician appears to have more to do with the physician' ability to communicate with the patient/ parents and demonstrate compassion and concern, than with the physician's clinical skills. In response, many physicians have resorted to defensive medicine in the hopes of averting a malpractice claim. Many doctors order additional tests, perform diagnostic procedures and refer patients for consultation when they do not believe that such action is clinically necessary.

Numerous studies have demonstrated that poor communication between physicians and parents/patients is the catalyst for most medical malpractice lawsuits. Communication and use of language is therefore critical. Good communication involves the use of lay terms and the avoidance of medical jargon [31].

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31

Environment and Early Developmental Care

Dominique Haumont

31.1 Introduction

The spectacular development of neonatal intensive care since the 1960s has allowed a drop in neonatal mortality of very-low-birth-weight (VLBW) infants from 50% to less than 15% in the last decade [1]. However 15 to 25% of the VLBW infants will present neurodevelopment impairment in the following fields: motor function, vision, auditory function, cognition, behavior, attention deficit and hyperactivity disorders, visual-motor integration and language [2, 3]. Compared to their term pairs there is substantial scientific evidence of altered early brain development [4]. These infants spend weeks and sometimes months in the neonatal intensive care unit (NICU), which is a quite different environment compared to what they would experience in utero. At this young age brain growth and development is particularly critical. The configuration of neurons is genetically predetermined, but the further organization and wiring of the neural circuits will depend on endogenous and exogenous stimulation. The existing evidence of interaction between environment and brain development has been extensively reviewed and better practices encouraged [5]. It is against the background of the potential harmful effects of the traditional NICU that developmental care and environmental strategies have gained more and more attention.

31.2 Early Developmental Care and Neonatal Individualized Developmental Care and Assessment Program

The term early developmental care (EDC) is confusing because it ranges from very simple interventions like light and noise control, positioning or non-nutritive sucking to the very complex Neonatal Individualized Developmental Care and Assessment Program (NIDCAP) [6]. The complex NIDCAP model is an early intervention program based on the observation of the preterm infant's behavior [7]. The theory behind NIDCAP is designated as "synactive theory". The concept derives from the interdependency, differentiation, modulation and regulation of five behavioral subsystems: autonomic, motor, state organizational (maturation of well-defined sleep and wake states), attentional/ interactional and self-regulatory. The assessment tool in NIDCAP is a formal and repeated observation of the infant behavior before, during and after a care. These observations describe the reactions of the baby towards the sensory inputs and his capacity of self-regulation. The developmental goals for each infant will be defined according to his individual skills and weaknesses. Individual care-giving plans and environmental adaptations will be recommended. As the NIDCAP implementation evolves in the unit the caregivers become more and more aware of the signs shown by the baby and handling is adjusted accordingly. The families are empowered in the caregiving and put in a central position. The competence of the NIDCAP observer has to be validated by certified NIDCAP trainers. The program requires a philosophy of care by the whole department and has to be strongly supported by medical and nursing directions.

Babies in the NICU are overwhelmed by sensory inputs. They perceive pain, thermal changes, movements, olfactory, vestibular, visual stimuli and social interactions with parents and caregivers. The effects of these sensory stimulations and the EDC recommendations are described below.

31.3 Sensory Stimulation and EDC Recommendations

31.3.1 Light

The development of the visual system has been fairly extensively investigated. The endogenous stimulation originating

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

from the spontaneous firing of neurons is important at different critical periods. The synchronous ganglion cell waves are targeting the different neurons to allow appropriate transmission towards the visual cortex. The protection of REM sleep is in this regard essential for normal development of ocular dominance columns. Light stimulation diminishes the action of the formative retinal waves [8, 9].

The preterm neonatal eye shows specific characteristics. Eyelid opening is related to the illumination of the NICU and the infant's developmental stage. The pupillary light reflex appears only after 30 weeks of gestation and mydriasis is physiological until 32 weeks. Light also acts as a stimulus for the production of free radicals in retinal membrane lipids. It has been suggested that the immature antioxidant status of the preterm infant might play a role on their susceptibility to retinal damage. Phototherapy and light are potential inducers of lipid hydroperoxides in babies receiving intravenous lipids [10].

31.3.2 Cycled Light

The endogenous biological clock of the circadian rhythmicity is located in the hypothalamic suprachiasmatic nuclei (SCN) at the base of the third ventricle. Input pathways entrained by light are relayed to the SCN, which will express output signals. These include temperature, cortisol, melatonin, sleep-wake-cycle, behavior and cardiovascular function. In utero, the fetus has a biological clock responding to maternal entraining signals. If the baby is born preterm he will be deprived of this circadian rhythm and submitted to an often chaotic NICU environment. Preterm infants have ultradian rhythms (period lengths much less than 24 hours), which parallels the maturation of the sleep pattern [11]. There is some clinical evidence that babies in cycled lighting grow and sleep better [5].

EDC recommendations include ambient lighting levels adjustable through a range of at least 10 to no more than 600 lux. No direct view of the electric light source or sun in the infant space. Separate adjustable procedure lighting up to 2000 lux shall be available to evaluate a baby or to perform procedures. Lipid infusion lines have to be protected from phototherapy light sources. The staff areas for preparation of medication or charting should be illuminated adequately. Windows provide an important psychological benefit for caregiving and tasks. Daylight should be controlled by shading devices to allow flexibility [12].

31.3.3 Noise

The acoustic stimuli of the fetus are fluid conducted, with predominance of the low frequencies of the mother's voice imbedded in circadian rhythms and less background noise. The preterm infant in the NICU receives air conducted sounds in all frequencies, sometimes very loud, unpatterned staff language signals with important background sounds and with no circadian rhythms [13]. It has been hypothesized that elevated noise levels and unpredictable background sounds are dangerous for the hearing development but also for the global brain development integrating all sensory channels. Clinical studies have shown the occurrence of hypoxic episodes and behavioral changes due to noise [14]. Nasal CPAP generates a high level of noise, which might be worrying [15]. Chaotic noise has also been shown to impact negatively on staff behavior.

EDC recommendations limit noise levels to 60 dB around the infants' space. Diurnal exposure to mother's voice should be encouraged. The acoustic environment should favor the baby's physiological stability and the needs of staff and parents [5, 12, 16].

31.3.4 Protection of Sleep

The term newborn sleeps around 70% of the time with about half of the time in well recognizable active sleep (AS or REM sleep) and quiet sleep (QS) patterns. Before 27 weeks it is not possible to identify a sleep pattern comprising AS and QS in the same cycle [17]. In the ontogeny theory, REM sleep is considered as a very intense cerebral activity playing an important role in brain maturation. The ultradian cycles of the preterm infant will progressively mature towards a circadian model of sleep/wake cycles. Maturation of sleep will be characterized by progressive change in the proportion of REM sleep towards a predominance of quiet sleep after term. The endogenous synaptic proliferation during REM sleep will prepare the brain for further refinement with exogenous stimulation.

There is growing evidence in animal and human research that supports the importance of sleep during early brain development. Animal studies on sleep deprivation showed structural and functional deterioration in several models. The need of endogenous synchronous waves during REM sleep has been well established for the development of synaptogenesis in the visual system [18].

EDC recommendation is to protect newborn sleep in the NICU [5].

31.3.5 Positioning and Handling

The flexed position and containment experienced by the baby in utero is a theoretical approach for subsequent positioning of the infant. Swaddling has been practiced for a long time and is associated with better sleep and diminished pain and stress response [19]. Babies with respiratory problems do often better in the prone position [20]. The strong neck extensor muscles have to be counterbalanced to support later motor development [21]. Healthy preterm infants have been shown to benefit from positioning in nests with flexed posture favoring more elegant movements and reducing frozen postures [22]. Routine cares like simple diaper changes can alter heart rate stability and oxygen saturation [23].

EDC recommendations are positioning of the baby in flexed position, with alignment of the head and minimal handling.

31.3.6 Kangaroo Mother Care (KMC)

KMC was started in 1978 in Columbia to support low birth weight infants in a low cost way [24]. The proven effectiveness and safety have since enlarged this practice in countries with no limited resources. The benefits attributed to KMC are decreased morbidity, improved weight gain, breastfeeding, neurobehavioral assessment and more mature sleep patterns. KMC is beneficial for the mother because it favors attachment and diminishes stress. Although less research about KMC in industrialized countries has been undertaken it is now current practice in the "Scandinavian model" [25].

EDC recommendations encourage KMC according to the baby's tolerance.

31.3.7 Pain and Stress

It is well known that newborn infants in the NICU undergo many painful procedures [26]. The fear of side effects of long-term exposure to narcotics on brain development and not recognizing pain and stress symptoms results in inappropriate pain relief in newborns. It has been shown that NIDCAP trained nurses, familiar with subtle clinical assessment were more likely to see clinical manifestations of pain [27]. On the other hand non-pharmacological interventions like oral sucrose, non-nutritive sucking, swaddling and facilitated tucking have been proven efficient [28]. Some units will claim an aggressive pain management with very liberal use of medication for medical procedures. However the first step in medical care is "do no harm" and therefore each procedure should be questioned. It is very likely that many routine interventions are historical and suppressible.

EDC recommendations consist of questioning the rationale of the procedure, carefully assessing the baby's behavior by skilled staff and adapting positioning, manipulation and medication.

31.3.8 Parental Presence

In the early days of neonatal intensive care universal nursing barrier was recommended and the babies were not supposed to need social interaction. It took many years before parental friendly attitudes were applied, although the importance of early relationships between the newborn infant and his parents has been shown a very long time ago [29]. Despite the general acceptance of unrestricted parental presence, standards of care are probably not applied everywhere due to local resistances.

EDC recommends intensive and if possible permanent parental presence and cooperation in the care giving of the infant. The impact of parents staying in the Nicu from admission to discharge is described in the Stockholm neonatal family centered care study. Length of stay was diminished and there was a reduced risk of moderate-to-severe bronchopulmonary dysplasia [30].

31.3.9 Oxidative Stress (OS)

Periventricular leukomalacia is the most important brain injury underlying the neurodevelopmental complications of preterm infants in later life. New insights into this disease relate the pathogenesis to incomplete vascular supply, deficient regulation of blood flow and the susceptibility of oligodendroglial precursor cells towards OS [31]. Many clinical situations in preterm infants will produce excessive free radical production: ischemia reperfusion injury, hypoxia and hyperoxia, inflammatory diseases, phototherapy and intravenous nutrition with long chain poly-unsaturated fatty acids [32, 33]. Their immature antioxidant defenses will put them at increased risk for OS. Any intervention in the NICU, which causes hypoxia, is potentially a source of OS. Intensive care procedures like intubation, chest drainage or intratracheal suctioning are well known to destabilize the baby. But even routine procedures like chest X-ray, changing electrodes, physiotherapy, excessive noise, weighing or even diaper change are accompanied by an acute decrease in transcutaneous oxygen tension [20].

EDC recommendations are to avoid any hypoxic event or unnecessary care.

31.4 Conclusions

We have summarized the neonatal practices where EDC might have an impact. We know that these recommendations are only partly applied in most of NICUs. Neonatal intensive care were framed like adult and pediatric intensive care units. But, in contrast to older patients, babies stay for a prolonged period and the caregivers have very few benchmarks for preterm infant normal behavior. These small patients have limited physical and undefined mental capabilities to express their suffering in ways that are understandable to their caregivers. Therefore they are totally dependent on the healthcare team to interpret, understand and intervene to alleviate their distress. The NIDCAP model addresses all these issues in a complex and holistic program. The literature overview demonstrating the effects of NIDCAP on neurodevelopmental outcomes is conflicting [6]. However, one can consider that caring for babies and paying attention to pain, stress and parental involvement is just a normal and humane behavior [34]. Sweden, which was a pioneer in NIDCAP training, has

Table 31.1 NIDCAP: 10 answers to early developmental goals

1.	Understand physiological organization of the preterm infant
2.	Diminish pain and stress
3.	Help parents in their difficulties: family centered
4.	Promote Kangaroo mother care
5.	Promote breastfeeding
6.	Stimulate parental presence
7.	Help the baby in his sleep organization
8.	Achieve a supportive patient-caregiver relationship
9.	Spread the spirit of humanization in the hospital
10.	Have a structured tool for change in the NICU

now been followed by several training centers in Europe, which support the diffusion of EDC [35, 36]. The advantage of the NIDCAP model is the consistency of the program and the global answers to our goals in EDC (Table 31.1).

Implementing EDC has also some side effects. One important aspect is the NICU architecture and design [12]. Many newly built NICUs have planned single rooms combining the requirements of intensive and special care in an EDC perspective. Putting respect, security, protection and privacy of the families in a central position will also impact attitudes in difficult situations like end-of-life decision making [37].

Introducing EDC in the NICU is moving away from traditional care towards a new culture characterized by respect of the preterm infant's physiology, diminishing pain and stress, with the parents as primary caregivers. It is likely that the construction of family wards and "couplet care" will allow optimal developmental care restoring the disrupted maternal continuity and her human monitoring capacities.

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32

Neonatal Pain: Neurophysiology, Recognition and Prevention

Carlo Bellieni

To address the question of pain in newborns, one must use indirect evidence from a variety of sources, and then make an informed guess. These sources include studies of behavior, anatomy and physiology. Evidence indicates that the experience of pain begins in the second trimester, well before the third and last trimester of human gestation [1] and that fetuses of that age can perceive pain [2].

32.1 Anatomy

First, we will take into account anatomical considerations. To feel pain, it is necessary for stimuli to involve neural connections between peripheral receptors and the spinal cord, upward transmission via the spinal cord to the thalamus, and from there to the outer cerebral layers. The development of the human nervous system is a progressive and ascending process, with the cerebral cortex the last region to develop. Thus, to experience pain, an intact system of pain transmission must be available.

32.1.1 Development of Connections

Peripheral receptors develop from the seventh gestational week [3]. Connections from the periphery to the spinal cord are formed early, at about 8 weeks [4]. C fibers begin to grow into the spinal cord at about 10 weeks. Early in gestation, after the tenth week of gestation, substance P and enkephalin-positive fibers appear [5]. From 13 weeks' gestation, the afferent system located in the substantia gelatinosa of the dorsal horn of the spinal cord develops [4, 6]. Spinothalamic connections start to develop from 14 weeks and are complete at 20 weeks' gestation, and thalamocortical connections are present from 17 weeks and completely developed at 26–30 weeks' gestation [7]. From 15 weeks, there is a subplate zone which lies beneath the cortex. This is a layer of neurons below the cortex that is specific to the fetus (see later). The thalamus plays a pivotal role in regulating the spinal-brainstem-spinal loops that mediate context-dependent descending facilitation or inhibition, coordinated through the key mechanisms underlying conscious- ness. In contrast to direct thalamocortical fibers, which are not visible until almost the third trimester, thalamic afferents begin to reach the somatosensory subplate at 20 weeks' gestation [8] and the visual subplate at 20–22 weeks' gestation [9]. Thalamic fibres penetrate the cortical plate from 24–28 weeks' gestation. From 20 weeks' gestation, peripheral receptors are present on the whole body [6, 10]. Recent studies have noted robust activation of the somatosensory cortex in preterm neonates exposed to tactile or painful stimuli, modulated by gestational maturity, postnatal age, sex, laterality, and sleep/ wake states [11, 12]. Cortical development starts only at about 17 weeks' gestation, but continues until long after birth. Synapses appear within the cortical plate from mid-gestation.

32.1.2 Subplate Zone and Cortex

The role of the cerebral cortex is crucial for pain sensitivity, but we should not ignore the role of the subplate in the development of sentience and consciousness. The subplate zone is a prominent, transient laminar compartment of the cerebral wall of the human fetus. The importance of the subplate as the main synaptic zone of the human fetal cortex is based on the rich input of "waiting" afferents from thalamus and cortex during the crucial phase of cortical target area selection. Recent neurobiological evidence shows that the subplate is an important site of spontaneous endogeneous activity, building a framework for development of cortical columnar

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

organization [13]. The conscious perception of pain requires peripheral pain receptors, connections to the spinal cord through an afferent system, fibers that connect the spine and the thalami, and most importantly, connections between the thalamus and the subplate zone or cerebral cortex. It is also important to know that pain impulses may be processed in other subcortical structures, including the hypothalamic pituitary system and amygdala (important for the emotional modulation of pain) [14]. The subplate zone expands considerably between 17 and 20 weeks and recedes after 30 weeks' developmental age [15]; from about 17 weeks, there is a shifting population of connections from the thalamus to this region. Neurons in the subplate zone initiate excitatory amino acid or peptide neurotransmission in the cortex, influencing the development of fetal cortical circuits [16, 17], while the cortical plate matures into the six layers of the cerebral cortex [18]. Differentiation of subplate neurons at 17–25 weeks' gestation produces five cellular subtypes whose distinct dendritic and axonal patterns correspond to different functional roles in development [19, 20]. From 16 weeks' gestation, pain transmission from a peripheral receptor to the cortex is possible and is certainly completely developed from 26 weeks' gestation. Assuming that activity in the cerebral cortex or subplate zone is necessary for consciousness, then for the fetus to be conscious of an external experience these regions need to be connected with incoming nervous activity. This starts to happen at about 16 weeks and puts an early limit on the timepoint at which it is likely the fetus might feel what is going on in its body or elsewhere [2]. Serotonin-releasing inhibitory descending pain fibers only develop after birth and it is therefore possible that preterm babies and fetuses feel more pain than small infants.

32.2 Stress Responses

32.2.1 Hemodynamic and Neuroendocrine Responses

Neonatal responses to painful stimuli have been evaluated using a variety of measures within three classes of responses (biochemical, physiological, and behavioral). Plasma catecholamine assays may provide the most valid and reliable measures of the biochemical responses of neonates to painful stimuli [21]. As early as 16 weeks' gestational age, the human fetus mounts a cerebral hemodynamic response to invasive procedures involving transgression of the fetal body, which is consistent with the "brain-sparing" effect [22]. Increased cerebral blood flow is not necessarily indicative of pain, as this response is thought to constitute a "brain-sparing" mechanism associated with hypoxia [23] and intrauterine growth restriction [24]. Nevertheless, fetal plasma concentrations of cortisol, -endorphin, and noradrenaline increase after intrauterine needling of the hepatic vein to a greater degree than of the umbilical cord [25, 26].

32.3 Long-Term Consequences

The evidence for adverse consequences on future neural development due to early exposure to noxious stimuli is increasing. Ruda et al studied an animal neonatal model of persistent hind paw peripheral inflammation. They found that, as adults, these animals exhibited spinal neuronal circuits with increased input and segmental changes in nociceptive primary afferent axons and altered responses to sensory stimulation [27]. Preterm infants who spend post-conceptional weeks 28 through 32 in a NICU are less mature in their pain response to heel prick than newborn premature infants of 32 weeks' post-conceptional age (PCA) [28]. Differences in these response patterns were strongly correlated with the number of invasive procedures experienced since birth, rather than other clinical factors (e.g. age, severity of illness, Apgar score, birth weight). These data suggest that repetitive pain and stress might alter the neurological response to pain, leading to altered neurobehavioral reactions to subsequent painful events [2]. According to Anand et al, repetitive pain in neonatal rat pups may lead to an altered development of the pain system associated with decreased pain thresholds during development [29]. Increased plasticity of the neonatal brain may allow these and other changes in brain development to increase the vulnerability of these animals to stress disorders and anxiety-mediated adult behaviour. Similar behavioral changes have been observed during the later childhood of ex-preterm neonates who were exposed to prolonged periods of neonatal intensive care: infants who are born prematurely or seriously ill are commonly exposed to multiple painful and stressful events as part of their prolonged hospitalizations and required medical procedures.

There is now evidence that early events not only induce acute changes, but that permanent structural and functional changes may also result [30]. Recent studies suggest that although early painful memories are not accessible to conscious recall, they might be encoded in "procedural memory" and lead to abnormal behavioral patterns or altered sensory processing in later life. Taddio et al demonstrated that infants circumcised at birth without analgesia showed a stronger pain response to subsequent routine vaccination than uncircumcised infants, or than those who underwent the procedure with analgesia [31]. It is becoming increasingly clear that neonatal painful experiences may leave a legacy of altered sensitivity to subsequent pain, perhaps for the entire life of the individual [32]. These findings should focus the attention of clinicians on the long-term impact of early painful experiences, and highlight the urgent need for developing therapeutic strategies for the management of neonatal and fetal pain.

32.4 Overcoming Procedural Pain

It is only since the end of the 1980s that neonatal pain has been widely recognized and treated. However, many different reasons for resistance still exist, as follows:

1. difficulty in defining pain [33];
2. lack of legal consequences for provoking pain;
3. confusion between understanding suffering and pain, leading to the false belief that drugs are the only answer to stress;
4. incorrect idea that newborns' fragile lives mean "fragile humanity", and they have a different moral status than older people;
5. self-censure on pain that is being provoked, to avoid being overwhelmed by it.

It is important to prevent, recognize and cure pain. Now we have the wherewithal to do so.

32.4.1 Prevention

Awareness of the full humanity of neonates In some cases neonates are still considered as having a different moral state than adults or older children [34]: for instance, end-of-life decisions may sometimes be taken considering not only the best interest of the neonate, but also parents' burdens and desires. Moreover, it has sometimes been argued that the apparent lack of consciousness in a neonate means a lack of personhood and of a full sensation of pain. Recognizing babies' full personhood means recognizing their right to a full well-being, including analgesia.

Manipulations and painful procedures must be reduced in number Babies have a lower pain threshold than older babies "because of the immaturity of their pain inhibition system" [35] and they undergo a mean number of 14 painful procedures/ day when in the NICU [36]. Any manipulation is potentially stressful if not painful: changes in temperature, light and noise can disrupt their behavioral states with harmful consequences.

Reduction of stress induced by clinical trials Clinical trials often provoke manipulations unnecessary to a baby's health. Moreover, studies of painkillers should never have placebo-treated babies as a reference group, because we cannot impose pain on anyone without his/her consent: parents should not be given the burden of deciding whether to enter their baby in a painful experiment, since pain must never be part of neonatal research (for a control group, a commonly recognized painkiller should be used). Unfortunately, preventing pain is not among the pre-eminent concerns of researchers: 75% of the studies considered in a recent review [37] showed that infants suffered pain during the research because placebo, no treatment or otherwise inadequate pain management was applied.

Provide newborns with an environment tailored to their needs Babies in incubators are exposed to very high electromagnetic fields [38], loud noises, disturbing lights, stressful manipulations and sometimes they are isolated from their family. We performed studies [39–41] to overcome some of these forms of environmental pollution, and effective developmental care, centered on the single baby and his/her family, is now recommended (Table 32.1) [42].

Table 32.1 Simple rules to approach neonatal pain

1. Address simultaneously the three sources of newborns' pain: procedural pain, parents' pain and doctors' and nurses' pain
2. Newborns' stress is often not only pain but also suffering: drugs cannot be the only answer
3. Newborns are full people: they need not only cures but full well-being
4. Reduce the number of stressful events and always apply good analgesia
5. Clinical research should not provoke pain and discomfort: alternative methods should be used and placebo use in studies on pain should be proscribed
6. Approaches like NIDCAP or sensorial saturation are an initial but good response
7. The perception caregivers have of a baby's pain may differ from the actual baby's experience: the use of validated pain scales is mandatory to be objective
8. Put pain treatment and developmental care in your basic medical know-how

32.4.2 Recognition

Carers should be able to recognize the main physical signs associated with pain. Most are non-specific: babies can cry for reasons other than pain, and pain is expressed through complex behavior. This does not mean that a crying baby should be ignored "because we are not sure" that he/she is crying for pain. On the contrary, this should alarm us as if it were pain: our duty is to exclude it and/or to treat it. Newborns show a distinct pattern of behavior to painful stimuli. This includes a wide range of expressions including screwing up the eyes, frowning, opening the mouth, extending the fingers, kicking, as well as clenching. For a thorough assessment, pain scales exist.

Chronic pain scales It is mandatory to monitor pain in order to prevent its occurrence, mainly after surgery or in ventilated babies. For this purpose, scales exist to evaluate the level of pain and stress in the previous period.

Acute pain scales Many scales exist in this field and are scarcely used. There are two reasons for this. First, the difficulty of applying scales where many items have to be assessed simultaneously [43]. Second, the lack of any point in assessing pain after it has occurred. Acute pain scales are useful for research purposes since they provide an objective way

Table 32.2 ABC acute pain scale

	Yes	Intermediate	No
Acuteness of the first cry	2	–	0
Crying constancy in time	2	1	0
Rhythmicity	2	–	0

of measuring the effectiveness of an analgesic drug. We recently described that the first cry after a heel prick is high-pitched if it is very painful. We also described that babies cry in 1 second bursts separated by short and regular pauses when pain is high [44]. We developed the ABC scale on this basis [45], which, unlike most other scales, can be used by the caregiver who is performing the procedure, without interrupting it to score pain, as it is uniquely based on recognizing the acoustic features of crying (Table 32.2).

32.5 Overcoming Parents' Pain/Anxiety

In the treatment of neonatal pain it is important to consider additional but often concealed parental pain, also because parents play an important role in the improvement of the baby. Caregivers should give adequate relationship-based care, and help couples to become true parents. In the NICUs this often happens through the foresight of the caregivers. Parents should be accompanied to meet their baby, gently encouraged to call him/her by name, and dedicate all the time needed to them; in some cases, they may require the help of a psychologist [46]. Parents' pain and stress will have two negative consequences: they may increase the difficulty of communication with physicians, leading to bad compliance and discussion; and will be transferred to the baby who may change his/her behavior accordingly.

32.6 Overcoming Healthcare Professionals' Pain/Anxiety

Healthcare professionals' pain/anxiety pain is often underestimated [47], but it is useful to consider that people work in a NICU side by side with death and pain on a daily basis, sometimes in difficult conditions. These people bear all this along with their own fears and anxieties. Much should be done to help them to find ways to cope with all this pain, to avoid their beautiful and important work becoming a routine, losing much of its usefulness, and sometimes reflecting upon the type of treatment they give babies [48]. Caregivers should be trained to have a positive vision of the baby, and encouraged to exploit any effort of the parents and any progress of the baby. Caregivers' anxieties can lead to unnecessary overtreatment or undertreatment [49].

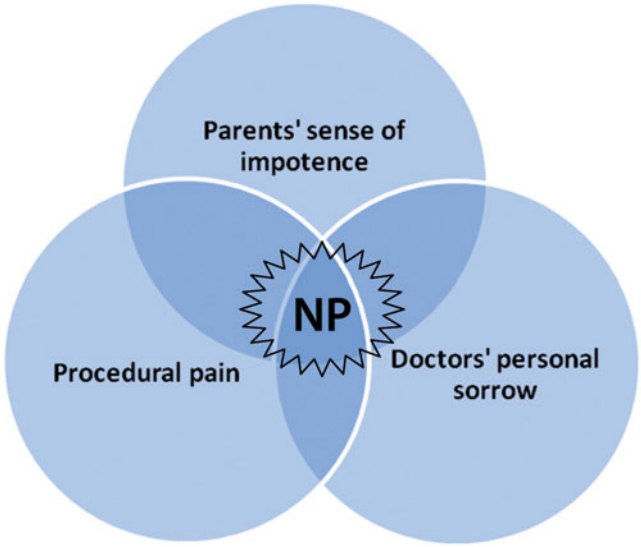

Fig. 32.1 Representation of neonatal pain (NP). Treating only one of the three legs of this tripod will be utterly insufficient

32.6.1 A Therapeutic Tripod

The therapeutic tripod represents an approach to pain relief. Neonatal pain can be relieved only by taking care of all of the legs (Fig. 32.1), considering non only procedural pain, but also caregivers' suffering [50–53]. Medical intervention should not be limited to drugs and technical procedures. What is required is "advocacy". Nurses and neonatologists should be advocates. They should also support parents to become active advocates, not to be frightened by their baby's apparent fragility, immaturity and lack of reactivity and to be able to interpret their baby's discomfort.

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33

Procedural Pain Management with Non-Pharmacological Interventions

Celeste Johnston, Ananda M. Fernandes and Marsha Campbell-Yeo

Neonates who are born preterm spend weeks in the neonatal intensive care unit (NICU) and undergo numerous painful procedures as part of their routine care [1]. In spite of evidence of negative effects and published guidelines on pain control, about one third of procedures remain unmanaged [2].

There are several difficulties with pharmacological interventions for procedural pain. Topical anesthetics have been shown not to be effective with heel lance or venepuncture, the most common painful procedures in neonates [3]. Systemic drugs, specifically opiates, have significantly slower clearance in neonates [4], and are also not necessarily effective for acute procedural pain [5]. Given the frequency of painful procedures in NICUs and the difficulties with pharmacological management, new approaches are required, and ones that have been tested will be reviewed.

33.1 Oral Sweet Solutions

Studies using sucrose started in the late 1980s and have included term infants as well as preterm and very preterm infants. Sucrose is the oral solution used most frequently and studies of it have been examined in two systematic reviews by Gaspardo et al and Tsao et al [6, 7] and one meta-analysis by Stevens et al [8]. These reviews concluded the positive efficacy of sucrose in reducing procedural pain in preterm neonates.

Concentrations of sucrose and have varied from 12–50%. Although one study reports a flat dose-response function [9], there seems to be a dose-response effect in the reduction of crying with increasing concentration of sucrose [10]. Volumes of sucrose administered in a single dose were 0.05 mL [11], 0.5 mL [12], 1 mL [13, 14] and 2 mL in all other studies. Sucrose has also been used in three repeated doses of 0.05 mL [11] and 0.1 mL [15] 2 minutes before heel lance. In the Cochrane review the recommended dose is 0.012–0.12 g (0.05–0.5 mL of 24% solution) and no statistically significant benefit in concentrations higher than 0.5 g (2 mL of 25% solution) was found [8].

Since sucrose was found to reduce composite measures of pain by approximately 20%, additional pain relief measures are recommended [8]. As will be described below, adding a pacifier improves the effect of sweet solutions.

Only a few studies have evaluated the occurrence of immediate adverse effects. Blood levels of glucose were monitored in infants who had 2 mL of 24% sucrose for three consecutive heel lances and no difference was found when compared to infants given a placebo [16]. The youngest infants in one report did have a higher incidence of immediate negative effects to sucrose, such as wretching, gagging, sustained tachycardia, and oxygen desaturation [17].

The effects of using sucrose routinely on consecutive days have been addressed but need further investigation. While one study of sucrose for all painful procedures in the first week of life in the NICU reported poorer neurodevelopmental scores with higher doses of sucrose [15], a secondary analysis found that this was the case in infants who had received more than ten doses over 24 hours [18]. Another study evaluating the routine use of sucrose over 4 weeks in the NICU did not find a higher incidence of intraventricular hemorrhage, nor neurobiological risk [19].

33.2 Non-Nutritive Sucking

Among non-pharmacological interventions, non-nutritive sucking (NNS), or pacifier use, was the first to be studied in the mid 1980s. Its use in term and preterm infants treated in neonatal intensive care and in minimal care shows that behavioral distress, namely percent time spent in the fussing and crying state, is reduced during and after heel stick [20]. Pacifier with sucrose was found to be more effective than no

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

intervention (mean PIPP [Premature Infant Pain Profile] score difference 1.92, $p < 0.0001$) and so was pacifier with distilled water (mean PIPP score difference 1.37, $p < 0.0006$) [21]. A meta-analysis of the effects of NNS on heart rate and transcutaneous oxygen tension ($TcPaO_2$) in studies from the past 30 years [22] found that NNS significantly reduced heart rate both in the presence and absence of painful stimulation and significantly increased $TcPaO_2$.

33.3 Breastfeeding

Several studies have also examined the possible benefits of supplemental breastmilk and or breastfeeding in full-term and older newborns. In a recent systematic review of 11 clinical trials, breastmilk and breastfeeding were shown to provide analgesia during routine procedural pain from heel stick and venepuncture [23].

Breastmilk alone does not appear to be as analgesic as sweet taste [24]. Only one study has examined breastmilk analgesia in infants younger than term gestation and found that it was not efficacious [24].

On the other hand, breastfeeding has been shown to be as effective as sucrose for the relief of procedural pain [25]. There is some evidence that breastfeeding may be superior to sucrose [26]. The benefits of glucose and breastfeeding may be cumulative when provided simultaneously [27].

Maternal presence appears to play a valuable role. Healthy full-term infants cried and grimaced less by 91% and 84% respectively when held during breastfeeding rather than swaddled during heel stick [28]. Breastfeeding and maternal holding with pacifier significantly reduced full-term infants crying behavior during heel stick when compared to the group being held by an assistant with pacifier [29]. During heel lance, being held by mother and breastfed was compared to being held by mother with pacifier and to being held by non-mother with pacifier [30]. In the first two conditions infants cried significantly less (33% and 45%) compared to being held by non-mother (66%, $p<0.01$ and $p = 0.03$, respectively).

33.4 Skin-to-skin Care or Kangaroo Care

Interest in kangaroo care (KC), described as the mother holding the baby naked with only a diaper in a prone up-right position against her bare breasts, for pain is recent. The effects of KC on infants' pain response were first studied in full-term infants [31] and were shown to reduce crying by 82% and grimacing by 65%, compared to infants who stayed in the crib during heel lance. Similar results have reported in fullterms [32].

The first study in preterm infants of 32–36 weeks gestational age was in 2003 [33] and other studies followed. These studies consistently show that KC significantly reduced PIPP scores during and after the painful procedure [34, 35]. In a trial comparing placebo, oral glucose and KC for heel lance [36], there were significantly lower pain scores in the KC group. The addition of rocking, singing and sucking in infants 32–36 weeks of gestational age, did not prove better than KC alone [37].

33.5 Containment/Facilitated Tucking and Swaddling

Containment refers to restricting the premature infants' motions by holding or using an arm to place the neonate's arms and legs near the trunk to maintain a flexed in utero posture with limbs placed in body midline [38]. It is also referred to as facilitated tucking [39]. The effects of facilitated tucking have been examined in preterm infants undergoing commonly performed tissue breaking procedures in the NICU and have been shown to diminish the magnitude of physiological and behavioural pain response [39, 40].

Similar to facilitated tucking in respect to containment and midline positioning, swaddling consists of wrapping the infant in a sheet or blanket [41]. The first study was published 20 years ago [42] where it was less efficacious in reducing pain than the pacifier. In preterm infants with a postconceptional age greater than 31 weeks, swaddling improved recovery from heel lance [43], but less so in infants with a postconceptional age of less than 31 weeks. A meta-analysis of four studies in Thailand reports that the effect size of swaddling compared to no intervention on pain scores during heel stick in term infants was 0.79 (95% CI = 0.53; 1.05) and in preterm infants was 0.53 (95% CI = 0.27; 0.80) [44]. Comparing containment to swaddling, there is little difference between their effects and therefore these interventions can be used interchangeably [38].

33.6 Rocking

Rocking, compared to pacifiers and routine care after heel lance, promoted arousal levels, while pacifiers promoted sleep and reduced heart rate [45]. Based on studies of rocking being effective in full-term neonates and on studies of simulated rocking promoting quiet sleep [45], simulated rocking was tested for pain in 85 preterm neonates, and rocking alone was no better than control unless coupled with sucrose.

33.7 Auditory Recognition/Music

The human fetus is thought to be capable of auditory perception by 29 weeks gestational age [46] and have the ability to remember auditory stimuli from their intrauterine environment [47]. In a crossover design study with infants 30–41 weeks

gestational age, music therapy alone consisting of intrauterine maternal pulse sounds with soothing music, music therapy combined with non-nutritive sucking, non-nutritive sucking alone and no intervention, were compared when used for 5 minutes after heel lance [48]. Music therapy alone had the strongest effect on neonates' heart rate. Butt and Kisilevsky [49], in a randomized cross-over design, exposed 16 preterm neonates to vocal or instrumental music for 10 minutes after the end of a heel lance. Infants above 31 weeks in the music group had a significantly more rapid recovery.

Similar findings were not observed in a recent study examining a recorded and filtered maternal "singsong" voice versus no voice during heel stick procedure [50]. These results may have been affected by the high volume of the recorded sound (70 db) or may indicate that familiar sound alone may not be sufficient to ameliorate the effects of a tissue breaking procedure in younger more immature infants.

33.8 Olfactory/Aromatherapy Recognition

There is now compelling evidence that both term and preterm infants remember, recognize and prefer smell that is associated with their intrauterine environment and their mothers, and that olfactory stimuli can provide infants with comfort and modulate pain response [51, 52]. These results were seen in both full-term [52, 53] and preterm (average 32.3 weeks gestational age) [51]. In a study that combined several sensory modalities, referred to as sensorial saturation, Bellieni [54] studied its effect on decreasing pain scores in preterm neonates in comparison to no treatment control, 10% glucose by mouth, sucking and combination of sucking and glucose. According to their definition of sensorial saturation, by simultaneously placing the baby in a flexed position with limbs brought to midline, talking to the baby while face-to-face, massaging the baby's face and back with baby oil scented hands, as well as orally administering 10% glucose, there was almost no pain (3/21). In a subsequent study with full-term neonates, Bellieni and colleagues [55] used 33% glucose in combination with sensorial saturation and essentially obliterated the pain response. Finally, this group tested the feasibility of training mothers to use sensorial saturation with the removal of baby oil to scent the hands, and found that they were as effective as highly trained staff and more effective than glucose and pacifier by two points on their pain score based on crying [56, 57].

33.9 Conclusions

Given the efficacy of numerous non-pharmacological interventions [58] for procedural pain in neonates and the difficulties with pharmacological agents in this population, for common painful procedures such as heel lance and venepuncture, non-pharmacological interventions should be the first choice in uncompromised infants [59]. They are cost-effective and easy to administer. Mothers are clearly implicated in breastfeeding and kangaroo care, but can also be included in other interventions [40, 57]. Initially, there is a requirement to train staff and parents in these methods, and it may seem easier to give a solution in an intravenous line than to coordinate care to coincide with parental visiting. Nevertheless, parents find pain the most distressing aspect of the NICU and also wish to actively participate in comforting their infant. These approaches are consistent with modern family centered care in neonatal units in which the best interests of the infant and family are put ahead of staff convenience.

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34

Neonatal Anesthesia

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34.1 Anesthesia

About 1.5 million fetuses or newborns are exposed to anesthetic agents each year.

Administration of anesthetic drugs, both with surgery and concomitant disease, can interfere with the developmental changes of neonatal life. It is mandatory for the pediatric anesthetist to know the pathophysiology of neonatal diseases in order to correctly apply the principles of neonatal anesthesia and surgery.

34.1.1 Preanesthetic Assessment

It is important to note details of the perinatal period and delivery and preoperative anesthetic evaluation should focus on the infant's transition from fetal to extrauterine life.

The intrauterine environment can interfere with growth and the ability of the neonate to adapt to extrauterine life. Maternal exposure to some drugs (e.g., phenytoin, alcohol, steroids) or abnormalities of placental blood flow may result in poor fetal growth or premature labour.

A complicated labor and delivery may result in respiratory, cardiovascular and metabolic instability for a variable length of time. Gestation and the infants' size also need to be taken into account.

The physical examination starts with the gestational age and the weight of the baby. A complete examination must be performed before withholding anesthesia from an infant, with particular attention for some parts of the body and systems, as summarized in Table 34.1.

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34.1.2 Intraoperative Management

Safe and effective intra-operative management of the newborn depends on an understanding of physiology and pharmacology. Technical aspects of monitoring and anesthesic equipment require consideration.

34.1.3 Induction of Anesthesia and Tracheal Intubation

The techniques for induction of anesthesia vary with the infant's size, gestation, medical status and surgical procedure.

A specific case is the infant with a full stomach. An "awake" or a rapid sequence intubation should be undertaken. However, the former approach is associated with significant morbidity (increased intracranial pressure, bradycardia, desaturation, etc.), and the latter is a real challenge, especially for small preterm infants.

Neonates with an expected difficulty in airway management (micrognathia, macroglossia, protruding maxilla, cleft palate, masses obstructing the airway) must not be paralyzed. Tracheal intubation can be performed with pharmacological sedation and with the infant breathing spontaneously. Topical anesthesia with lidocaine on the vocal cords can facilitate tracheal intubation.

The anatomical peculiarities of the neonate and subsequent anesthetic implications are summarized in Table 34.2.

The Miller-0 straight laryngoscope blade is the recommended device for visualizing the airway for tiny neonate, preterm or of low birth weight, and the Miller-1 is used for term neonates. Uncuffed 3.0–3.5 mm endotracheal tubes are usually appropriate for tracheal intubation of term newborns. A tube that fits too tightly can damage the subglottic mucosa, causing edema and postoperative stridor or possibly subglottic stenosis. A leak around the tube during manual positive breaths (about 20 cmH_2O pressure) ensures that the

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Table 34.1 Pathophysiology and anesthetic implications in the newborn

Organs / Systems	Pathophysiology	Anesthetic implication
Head and Neck	Micrognathia CHARGE syndrome	Possible difficulties in airway management Peculiar embryology and anatomy in preterm and term infants
Respiratory	Immaturity of control mechanism More compliant chest wall 10–25% type I fibers Surfactant deficiency Oxygen toxicity	Respiratory depression in case of hypoxia Airway collapse Predisposition to respiratory fatigue Respiratory distress syndrome (RDS) Bronchopulmonary dysplasia (BPD) development
Cardiovascular	Hypoxemia or acidosis	Return to fetal circulation, persistent fetal circulation (PFC), persistent pulmonary hypertension of the newborn (PPHN)
CNS	Fragile vessels Immaturity of cerebral blood flow	Increased risk of hemorrhage Hypoxia, acidosis, seizures may disrupt autoregulation
Renal	Immaturity	Lost of electrolytes
Metabolic	Active transplacentar delivery of calcium and transient hypoparathyroidism Delay in normal feeding	Hypocalcemia Hypoglycemia

Table 34.2 Airway management in the newborn

Anatomy of the newborn	Action during tracheal intubation
Prominent occiput and large head	"Sniffing position" without the additional support of a pillow under the head neck
Larynx is cephalad (C4) and anterior	Extension of the neck may impede visualizing of the vocal cords, gentle pressure with finger can facilitate larynx view
Epiglottis	Large and with "ʊ form", difficult visualization of the vocal cords
Narrow nostril and large tongue	Obligate nasal breathing, airway obstruction, possible bleeding
Cricoid cartilage	Is the narrowest airway of the newborn, small and uncuffed tubes

endotracheal tube is not too large. Ensuring that the appropriate depth in the trachea of the newborn is reached also requires care. The distance from the vocal cords to the carina in the term infant is about 4 cm.

34.1.4 Intraoperative Fluid Therapy

Intraoperative fluid therapy has four components:

1. *Maintenance of fluid* Normal fluid losses consist mainly of insensible water loss from the respiratory system, evaporation from the skin, urinary and fecal loss. The normal requirement is 100 mL/kg/day.
2. *Fluid deficits* These are caused mainly by preoperative fasting or excessive gastrointestinal losses without parenteral replacement. The deficit is calculated by multiplying the hourly maintenance requirement by the number of hours since the last fluid intake.
3. *Third space fluid loss* Surgical trauma can result in translocation of extracellular fluid from the intravascular space into the interstitial space (edema in the bowel wall and mesentery during intra-abdominal surgery). Guidelines for the replacement of third space losses include:
   a. superficial surgery: 1–2 mL/kg/hour
   b. abdominal, chest or hip surgery: 3–4 mL/kg/hour
   c. extensive abdominal surgery: 6–10 mL/kg/hour or more.

   Third space losses must be replaced with an isotonic or iso-osmotic solution such as normal saline solution, lactate Ringer's solution, or other balanced salt solutions.
4. *Other fluid losses* These include those due to gastric or endotracheal suction or removal of gastric intestinal fluid or drainage of an ileostomy, diarrhea or excessive sweat losses. In these cases the electrolyte content of the fluid losses should be measured to determine replacement fluids.

A normal urine output (0.5–2 mL/kg/hour) indicates normal hydration. Serum osmolarity is also a useful monitor of electrolyte and fluid therapy.

Standard hemodynamic intraoperative monitoring contributes to assessing the adequacy of fluid therapy.

As with older infants, the decision to deliver blood during surgery depends on the underlying and current cardiorespiratory status, ongoing blood loss, anticipated further blood loss, and baseline hemoglobin. Transfusion of other blood components should be guided by a combination of laboratory studies and clinical status.

34.1.5 Anesthetic Drugs: Inhaled and Intravenous Agents

The aim of anesthesia is to provide insensibility to pain during surgery. Anesthesia may be provided by regional or general anesthesia or the combination of both. General anesthesia can be delivered using both inhaled and intravenous drugs for a variety of surgical procedures.

Minimum alveolar concentration (MAC) defines the anesthetic depth for inhaled agents at which 50% of patients respond to painful stimulus with movement. It has been found that MAC is considerably less in preterm than in full-term infants and that 1 MAC of inhaled drugs results in 20-30% reduction in arterial pressure. Sevoflurane, newer inhaled agent, afford a rapid induction and emergence from general anesthesia and is now widely used for anesthesia in infants. Moreover, sevoflurane possesses less airway irritability than isoflurane and desfurane, and it can be used for inhalational induction and in infants with bronco pulmonary dysplasia. The traditional mask induction of anesthesia is accomplished with incremental increases in sevoflurane up to 8%, in a mixture of oxygen and air. Maintenance of anesthesia is performed with 1 MAC of sevoflurane, and it can be reduced in case of opioid administration or combination with regional anesthesia.

Intravenous drugs for anesthesia may include opioids, benzodiazepines, propofol, ketamine, barbiturates, and dexmedetomidine which is recently introduced in clinical practice. Opioids possess analgesic and sedative properties and they are usually combined with hypnotic drugs as propofol or benzodiazepines. Pharmacokinetic of fentanyl has been studied in infants and the elimination half time has been found to be from 6 to 32 hours, which is significantly longer than in adults. Remifentanil, a new synthetic short acting opioid, is rapidly inactivated by esterases in blood and tissues and because of its short half-life is administered by continuous infusion. For this reason is now largely used in all ages and is a suitable opioid even in small infants [1]. Doses of some anesthetic drugs are summarized in Table 34.3.

34.1.6 Anesthesia for Preterm and Ex-Premature Infants

Preterm and ex-premature infants undergoing elective surgery are more likely to encounter perioperative apnea than term infants. Factors predisposing premature infants to apnea include hypoglycaemia, hypoxia, hyperoxia, sepsis, anemia, hypocalcemia and pharmacologic effects of general anesthesia on the immature respiratory centres. As a consequence, postoperative monitoring is mandatory for the 24 hours following anesthesia.

Preterm and ex-premature infants may have dramatic responses to narcotics and potent inhaled anesthetics. The benefit of providing adequate anesthesia and analgesia must be carefully balanced against the significant risk of cardiorespiratory depression in these vulnerable patients.

Table 34.3 Principal drugs and doses used for neonatal anesthesia

	Induction	Maintenance
Sevoflurane	Incremental, up to 8%	0.5–2 MAC
Ketamine	1–2 mg/kg	Not recommended
Propofol	2–5 mg/kg	50–200 μg/kg/min
Thiopental	4–6 mg/kg	Not recommended
Midazolam	0.05–0.2 mg/kg	100–200 μg/kg/h
Fentanil	0.5–3 μg/kg	Up to 100 μg/kg for cardiac or major surgery
Remifentanil	0.1–1 μg/kg/min	0.1–1 μg/kg/min

34.1.7 Regional Anesthesia

Epidural analgesia in combination with light general anesthesia is a useful alternative for infants and neonates undergoing major surgery, avoiding the adverse effects related to systemic administration of opioids and other agents. Apart from providing good intraoperative and postoperative analgesia, epidural blockade has beneficial effects on the humoral, metabolic and hemodynamic responses to surgery and may improve postoperative respiratory performance.

The complication rate of epidural analgesia performed by an experienced operator is low. Caudal epidural anesthesia remains the most frequently performed regional anesthesia technique for infants and children. It is a popular "single shot" technique characterized by a high level of efficacy and safety.

Because of the lower levels of plasma protein α1-acid glycoprotein, albumin, and lower bicarbonate reserves, neonates are risk of local anesthetic toxicity, e.g. cardiac dysrhythmia or respiratory arrest, which are more likely than convulsions in neonates and infants. This can be avoided using bolus doses and infusion rates that are within recommended guidelines and by taking into account pharmacokinetics of local anesthetics in neonates. The low intrinsic toxicity of new local anesthetics like ropivacaine and levobupivacaine makes them ideal as local anesthetic for pediatric use.

The use of spinal anesthesia for hernia repair in premature infants with or without respiratory distress syndrome (RDS) is a safe and satisfactory alternative to general anesthesia. Although a review by Craven in The Cochrane Database of Systematic Reviews [2] reported no reliable evidence on the incidence of apnea, bradycardia, or oxygen desaturation in ex-preterm infants for spinal anesthesia compared to general anesthesia, spinal anesthesia may be considered a safe alternative for the avoidance of general anesthesia related risks. Further studies are needed to confirm whether there are real additional benefits for spinal anesthesia for small infants.

34.1.8 Anesthesia Outside the Operating Room

Sedation outside the operating room or in remote locations is increasingly called, particularly for radiologic procedures and medical interventions. Providing anesthesia in locations far from the operating room requires familiarity with the procedures as well as careful and extensive planning and resources in order to manage life-threatening situations. Some of remotes locations that may require sedation include dental clinics, gastroenterology suites, cardiology sites, plastic surgery centres, radiology suites, oncology areas. It is advisable to delegate a team of anesthetists that is committed to providing off-site anesthesia care. Each member of the anesthesia team should rotate regularly through the different off-site areas to maintain a familiarity not only with the procedures and personnel but also with the particular anesthesia demands unique to each area. A standard anesthesia cart should be at each anesthesia site, fully stocked with essential medications, necessary adjuvant equipment, a variety of oral/nasopharyngeal airways, spare Ambu bag, laryngoscope handles and blades, endotracheal tubes, alternate airway devices including laryngeal mask airways (LMAs), suction catheters, and intravenous supplies.

The most important topic to be considered before performing a sedation outside the operating, especially in infants younger than 1 months of age, are:

- History of ongoing apnea or prematurity with post-conceptional age less than 60 weeks
- Respiratory compromise and abnormal airway
- Congenital cardiac disease or unstable cardiac status
- High-risk procedure
- Inadequate qualified personnel or inadequate anesthesia and resuscitation equipment.

A clear understanding of the definition of sedation is mandatory to recognize when the child has progressed to a deeper level of sedation than anticipated and is at increased risk (i.e., from moderate sedation to deep sedation or from deep sedation to general anesthesia). Recognition of this transition allows escalation of monitoring and care to avoid complications.

The following are the definitions of the American Academy of Pediatrics (AAP) and the American Society of Anesthesiologists (ASA) [3], strong considering that infants may quickly move from one level of sedation to another.

Minimal sedation A drug-induced state during which patients respond normally to verbal commands.

Moderate sedation A drug-induced depression of consciousness during which patients respond to light tactile stimulation. No interventions are required to maintain a patent airway, and spontaneous ventilation is adequate.

Deep sedation A drug-induced depression of consciousness during which patients cannot be easily aroused but respond after painful stimulation. The ability to independently maintain ventilatory function may be impaired. Patients may require assistance in maintaining a patent airway and spontaneous ventilation may be inadequate.

General anesthesia A drug-induced loss of consciousness during which patients are not arousable, even to painful stimulation. The ability to independently maintain ventilatory function is often impaired. Patients often require assistance in maintaining a patent airway, and positivepressure ventilation may be required because of depressed spontaneous ventilation or drug-induced depression of neuromuscular function. Cardiovascular function may be impaired.

Several anesthetics drugs [4, 5] for sedation in infants are summarized in Table 34.4.

Table 34.4 Sedative drugs for infants

Drug	Doses	Comments
Midazolam	0.5–0.75 mg/kg PO or ER 0.025-0.05 mg/kg IV 0.02-0.03 mg/kg intranasal	Possible paradoxical effects. Doses must be reduced in case of concomitant opioid administration
Ketamine	1–2 mg/kg IV 3–4 mg/kg IM 4–6 mg/kg PO or ER	Possible laryngospasm, apnea, agitation, hallucinations. Anticholinergic to control secretions. Frequent associated tachycardia, hypertension, and bronchodilatation. No antagonist available
Nitrous oxide	50% in 50% oxygen for "minimal sedation"; up to 70% for moderate sedation	Requires specialized equipment for delivery, monitoring, and scavenging. When used alone or with local anesthesia is considered "minimal sedation." Contraindications include respiratory failure, altered mental status, otitis media, bowel obstruction, and pneumothorax
Sevoflurane	1–2 MAC inhalational	Safely used for MRI and TC. It has little toxicity and its hemodynamic and respiratory depressive effects are moderate and well tolerated
Naloxone	0.01–0.1 IV or IM, may be repeated every two minutes	Specifically antagonizes opioid effects. Adverse reactions: nausea, vomiting, tachycardia, hypertension. Reversal after long-term opioid use may lead to acute withdrawal
Flumazenil	0.01–0.02 IV, may be repeated every one minute	Specific benzodiazepine antagonist. Does not antagonize opioids or other sedatives. Re-sedation may occur in 1 hr. Prolonged observation (2 hr) required. Not for routine sedation reversal

PO per os, *ER* endo-rectal, *IV* intravenously, *IM* intramuscularly, *MAC* minimum alveolar concentration.

34.1.9 Neonatal Anesthesia and the Risk of Neurotoxicity

A great deal of concern has recently arisen regarding the safety of anesthesia in infants. There is a convincing preclinical evidence that commonly used anesthetics are neurotoxic to the developing brain and may cause neurobehavioral abnormalities [6-8]. The clinical relevance of anesthetic neurotoxicity is an urgent matter of public health and two large-scale clinical study are currently underway to further address this issue. The GAS Study is an international randomized study comparing two anesthetic techniques, general sevoflurane anesthesia and regional anesthesia, in 660 infants undergoing inguinal hernia repair. The neurodevelopmental outcome will be at age 2 and 5 year. The results from this study should contribute significant information related to anesthetic neurotoxicity in neonates.

34.2 Analgesia

Interest in newborn and preterm pain has greatly increased during the last three decades, including management of pain associated with intensive care and surgery. In the past, pain relief in these very vulnerable subjects has been neglected, reflecting the current beliefs and a resistance to change practice.

34.2.1 Effects of Pain

The fetus is able to respond to noxious stimuli by increasing stress hormone levels and cerebral blood flow, whereas fentanyl dampens the stress response by preterms undergoing surgery [9]. These observations support the notion that autonomic and metabolic reactions are triggered before pain pathways are mature.

Between 17 and 25 weeks of gestational age, several neuronal cells types and dendritic and axonal networks differentiate from subplate neurons, assigned to guide the development of cortical and thalamic structures. Because of nervous system plasticity, repetitive noxious stimuli reaching the subplate neurons may result in the formation of permanent abnormal synapses and hyperactivity of pain responses, resulting in possible future behaviour disorders.

34.2.2 Pain in the Neonatal Intensive Care Unit

Though guidelines to manage pain in the neonatal intensive care unit (NICU) have been issued [10], many newborns undergo repetitive noxious procedures without adequate pain relief. Acute pain assessment can be difficult, because of difficulties in (a) the discrimination of pain from other causes of distress and (b) recognizing pain cues in the sickest babies. Furthermore, signs of pain may not be apparent because of limited energy reserves and exhaustion in the sickest infants. Other obstacles to pain management can be fear of respiratory depression, routine practice, old beliefs and lack of validated prolonged pain assessment scales [11].

Beyond procedural, perioperative and disease-related pain relief, sedation in mechanically ventilated neonates has been advocated, aiming at promoting stress reduction, blood pressure stability and ventilator synchrony, leading to decreased cerebral injury and death. In 2004, the NEOPAIN multicenter trial evaluated 898 ventilated preterm newborns randomized to receive morphine or placebo. The study did not demonstrate any difference in the incidence of intraventricular hemorrhage, periventricular leukomalacia and death between the groups, while the morphine group had more hypotension, prolonged ventilation and feeding intolerance [12].

Although opioids cannot be currently recommended for routine sedation in ventilated neonates, they should be routinely administrated during surgery, invasive procedures and inflammatory diseases, on the basis of pain evaluation and clinical judgement [13].

34.2.2.1 Opioids

The most commonly used opioids in NICU are morphine and fentanyl. Morphine shows an onset time of 5 minutes and a peak effect at 10–30 minutes. The effect lasts 3–8 hours in neonates. Clearance reaches adult values at 6 months. The relative permeability of the newborn blood-brain barrier facilitates the penetration of morphine into the central nervous system compared to older subjects. Morphine is administered for perioperative analgesia, but has not proved to be so effective for procedural acute pain. Usual intravenous dosage in infants receiving ventilatory support is 50–100 μg/kg as a single dose, followed by an infusion of 10–30 μg/kg/h. Lower dosages are adequate for lower gestational ages and small incremental doses (25–50% of the usual) are advised for non-ventilated infants.

Fentanyl is more lipid soluble and has a potency 100-fold that of morphine. The peak effect is reached by 5–15 minutes and its duration is 1–2 hours. Fentanyl is less sedating and has minimal cardiovascular effects. It is suitable for hemodynamically unstable patients. Nevertheless, disadvantages of fentanyl are the rapid development of tolerance and chest wall rigidity, which is particularly problematic for non-intubated infants. Fentanyl is usually administered intravenously at a dosage of 1–3 μg/kg as a single dose, followed by an infusion of 0.5–2 μg/kg/h.

Tolerance develops more rapidly with fentanyl (3–9 days) than with morphine and with a continuous infusion than with intermittent doses. Non-pharmacological measures and switching from opioids to sedatives (when appropriate) can

reduce the risk of developing tolerance. Opioid tapering protocols should be implemented in every NICU.

Codeine is administered only by the enteral route, needing the enzyme CYP2D6 to be metabolized to morphine. Poor and intermediate metabolizers can undergo dose-related toxicity or therapeutic failure. Ultrarapid metabolizer breastfeeding mothers expose their babies to the risk of life-threatening respiratory depression [14].

Tramadol is an analogue of codeine, acting both as μ-receptor agonist and monoamine reuptake inhibitor. CYP2D6 metabolizes tramadol to O-demethyl tramadol, which shows a much higher affinity for μ-receptors than the parent compound. In term neonates, CYP2D6 activity seems to be equal to adult slow metabolizers and reaches adult activity level at 44 weeks. Tramadol is administered at the dose of 2–3 mg/kg as a bolus and 5–8 mg/kg/day as a continuous infusion [15].

34.2.2.2 Paracetamol

Paracetamol is a very common analgesic in infancy, but in neonates poor pain relief is reported after its administration for painful procedures, probably indicating its inadequacy for this type of pain. Newborns are relatively protected from paracetamol hepatotoxicity, because of a lower CYP450 level and lower glucuronide/sulphate metabolism ratios [16]. Increased unconjugated hyperbilirubinemia reflects the hepatic conjugating ability and can be a marker of reduced paracetamol clearance. The relative bioavailability of rectal versus oral formulations is higher in neonates and approaches unity, while the rate of duodenal absorption is slower and the rate of rectal absorption is slower and more erratic in neonates compared with older infants. To reach a target plasma concentration of 10–20 μg/mL, a loading dose is recommended. Doses should be adjusted in relation to weight, gestation, postnatal age and route of administration. Term newborns should not receive more than 60 mg/kg/day of paracetamol at 6 hourly intervals, whereas preterms should receive lower doses (30–40 mg/kg/day) at longer intervals (12–8 hours) [17]. Intravenous paracetamol can be given as opioid sparing drugs either alone or in association with an opioid. The full dosage ranges from 30 to 60 mg/kg/day and should not be continued for more than 4 days.

34.2.2.3 Ketamine

Ketamine is a "dissociative" drug, providing anesthesia, analgesia and amnesia, while displaying a safe respiratory and hemodynamic profile. The new S(+) ketamine seems to be better than the older racemic compound. Drug action is mediated mainly by N-methyl-D-aspartate (NMDA) receptor antagonism. An intravenous dose of 1–2 mg/kg exhibits an onset time of 1 minute, a peak effect by 5–10 minutes and a duration of 30–60 minutes. In NICU procedural pain, ketamine reduces the discomfort associated with tracheal suctioning [18]. Recently, the Food and Drug Administration have invited the anesthesia community to address the issue of the potential neurotoxicity of anesthetic agents in neonates on the basis of their neurodegenerative effects on the developing brain in experimental trials [19]. NMDA receptor antagonists and γ-aminobutyric acid (GABA) agonists in doses and durations of exposure exceeding those clinically used, induce neuronal injury and death in the brains of juvenile rodents. Most of these studies relate to ketamine, as initial observations had suggested a possible role for NMDA antagonists as neuroprotective agents. However, subsequent studies have shown neurotoxic effects. The applicability of these reports to clinical situations needs further confirmation.

34.2.2.4 Midazolam

Benzodiazepines are the most popular sedative drugs used in children, often combined with opioids. They share the same GABA-ergic mechanism of action with propofol and barbiturates, resulting in hyperpolarization of the neuronal membrane and producing anxiolysis, amnesia, sedation, muscle relaxation and anticonvulsant effects. Potential adverse effects are hypoventilation/apnea and hypotension. Midazolam is characterized by a faster onset and shorter duration than other drugs such as diazepam and lorazepam. The immaturity of enzyme systems involved in the metabolism of the drug (CYP450 and glucuronyltransferase) are linked to gestational and postnatal age, organ dysfunction and drug interactions, and explain the marked interpatient variability in dosage requirements to produce sedation in critically ill pediatric patients [20]. The use of midazolam in NICU has been recently reassessed on the grounds of both clinical and experimental observations. In 1999 the NOPAIN trial showed a higher incidence of adverse neurologic events and death in a midazolam group compared with morphine or placebo groups [21]. The potential of midazolam to induce apoptotic neurodegeneration in the brain, while failing to sedate and sensitize cutaneous reflexes in neonatal rats, has raised concerns about the safety of midazolam during the first days of life. Midazolam infusions for sedation in NICU are, however, used.

34.3 Conclusions

Physiology and pathophysiology of the neonatal age and the pharmacology of anesthetic and analgesic drugs form the basis for anesthesia management of the newborn. Small infants, preterms and neonates with complex syndromes can be safely managed but only by experienced pediatric anesthetists.

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35

Neonatal Care in the Delivery Room: Initial Management

Tara M. Randis

35.1 Introduction

Ancient accounts of neonatal resuscitation strategies may be found in the Old Testament, the Talmud, and early writings of Hippocrates [1]. Mouth-to-mouth resuscitation of newborns was described as early as 1472 in a textbook on childhood diseases written by Bagellardeus [2]. While, some of these early resuscitation practices were truly revolutionary, the vast majority reflected a limited understanding of the dramatic physiologic changes that take place during birth. Techniques including swinging the infant upside down, immersion in cold water, electric shock, shaking, yelling, slapping and even insufflation of tobacco smoke into the rectum were widely practiced as recently as the early 20th century [1–3]. In 1953, Virginia Apgar wrote the following regarding the resuscitation of newborn infants:

> Seldom have there been such imaginative ideas, such enthusiasm, and dislikes, and such unscientific observations and study about one clinical picture. There are outstanding exceptions to these statements, but the poor quality and lack of precise data of the majority of papers concerned with infant resuscitation are interesting. [4]

Over the past several decades, an improved understanding of neonatal transition to extrauterine life accompanied by major advancements in medical technology have led to dramatic improvement in the care of the compromised newborn.

Recognition of the growing need for standardization of delivery room practices has led to the development of national committees dedicated to the establishment of consensus guidelines for newborn resuscitation. A global movement towards the practice of evidence-based care in the delivery room emerged and in 1992 the International Liaison Committee on Resuscitation (ILCOR) was established to provide a forum for liaison between resuscitation organizations in the developed world [5]. Importantly, this committee not only provides recommendations regarding the care of the newborn, but also has acknowledged ongoing controversies and identified areas in which additional research is needed. In addition, the training of caregivers in the delivery room has become the focus of recent clinical investigations, as innovative educational strategies such as video recording of resuscitations and simulator-based teaching become an integral part of many pediatric training programs [6, 7].

35.2 Delivery Room Preparation

Successful resuscitation hinges upon preparedness. Nearly 10% of newborns require some assistance to begin breathing at birth and approximately 1% will require extensive resuscitation. Personnel trained in basic resuscitation skills (including initiation of positive pressure ventilation and chest compressions) should be present at every delivery. A person trained in Advanced Life Support techniques should be readily available for low-risk deliveries and present in the delivery room for those considered high-risk. Published recommendations regarding the necessary equipment and drugs for resuscitation of the newly born infant exist and delivery rooms should be stocked accordingly (Table 35.1)[8]. Communication with maternal care providers is critical, as a thorough maternal history will often identify those neonates most likely to require resuscitation. In 2008, Aziz et al [9] published a prospective, clinical trail identifying both ante-partum and intra-partum factors that were strongly associated with the need for positive pressure ventilation in the delivery room. These included multiple pregnancy, maternal infection, maternal hypertension, oligohydramnios, preterm delivery, breech presentation, meconium stained amniotic fluid, non-reassuring heart rate patterns, and emergency C-section.

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Table 35.1 Neonatal resuscitation supplies and equipment

Suction equipment	Bulb syringe Mechanical suctioning and tubing Suction catheters (5F or 6F, 8F, 10F, 12F or 14F) 8F feeding tube and 20 mL syringe Meconium aspirator
Bag and mask equipment	Device for delivering positive-pressure ventilation (capable of delivering 90–100% O_2) Face masks, newborn and premature sizes (cushion-rim masks preferred) Oxygen source with flowmeter (flow rate up to 10 L/min) and tubing Compressed air Oxygen blender with flowmeter Pulse oximeter and probe
Intubation equipment	Laryngoscope with straight blade, No. 0 (preterm) and No. 1 (term) Extra bulbs and batteries for laryngoscope Endotracheal tubes (sizes 2.5, 3, 3.5, and 4 mm ID) Stylet (optional) Scissors Tape or securing device for endotracheal tube Alcohol sponges CO_2 detector or capnograph Laryngeal mask airway (optional)
Medications	Epinephrine: 1:10,000 concentration (0.1 mg/mL) [3 mL or 10 mL ampules] Isotonic crystalloid (normal saline or Ringer's lactate) for volume expansion Sodium bicarbonate: 4.2% (5 mEq/10 mL) [10 mL ampules] Naloxone hydrochloride: 0.4 mg/mL [1 mL ampules], or 1.0 mg/mL [2 mL ampules] Dextrose 10%, 250 mL Normal saline for flushes Umbilical vessel catheterization supplies (sterile gloves, scalpel or scissors, antiseptic prep solution, umbilical tape, umbilical catheters, 3.5F, 5F, three-way stopcock) Syringes 1, 3, 5, 10, 20, 50 mL Needles, 25, 21, 18 gauge, or puncture device for needless system
Miscellaneous	Gloves and appropriate personal protection Radiant warmer or other heat source Firm, padded resuscitation surface Clock with second hand (timer optional) Warmed linens Stethoscope Tape, ½ or ¾ inch Cardiac moniter and electrodes or pulse oximeter an probe (optional for delivery room) Oropharyngeal airways (0, 00, 000 sizes or 30, 40, and 50 mm lengths)
For very preterm babies (optional)	Compressed air source Oxygen blender to mix oxygen and compressed air Pulse oximeter and oximeter probe Reclosable, food-grade plastic bag (1 gallon size) or plastic wrap Chemically activated warming pad Transport incubator to maintain baby's temperature during move to the nursery

From [8], with permission of AAP.

Those infants born after a full-term gestation, with no evidence of meconium or infection, with spontaneous breathing and good muscle tone are unlikely to require resuscitation and therefore may be dried and immediately placed upon the mother's chest [8]. If an infant does not meet these criteria, then the health care practitioner should proceed with the initial steps of resuscitation including:

1. Placing the infant under a radiant heat source
2. Proper positioning/clearing of the airway
3. Drying and, if necessary
4. Stimulating the infant to elicit effective respirations.

35.3 Positioning and Clearing the Airway

Placement of the newborn on his or her back under a radiant warmer with the head in a slightly extended position will open the airway. If respiratory efforts are present but ineffective (retractions, inadequate chest wall movement or poor air entry), the airway may be obstructed.

Secretions should be cleared first from the oropharynx and then the nasopharynx with either a bulb syringe or suction catheter. Care must be taken to avoid deep or vigorous suctioning as this may result on laryngeal spasm and/or vagal bradycardia.

35.4 Stimulation

Most infants cry spontaneously upon delivery and establish regular respiration by 1 minute of age. If this does not occur, the majority of infants will respond to tactile stimulation such as gentle rubbing of the back, as occurs with drying of the infant, or gently flicking the bottom of the feet. Failure of the infant to respond to these measures indicates the infant may be experiencing secondary apnea, and will require positive pressure ventilation. Resuscitative measures beyond these initial few steps are discussed in detail in subsequent chapters.

35.5 Thermal Stability in the Delivery Room

Fetal temperature in utero is tightly regulated. Fetal metabolism results in a net production of heat that is removed by placental blood flow and is ultimately dispersed through the mother. As a result, there is a slight temperature gradient, with fetal temperature approximately 0.5°C greater than the maternal core temperature [10]. Upon parturition, the neonate rapidly loses heat through a variety of mechanisms as the ambient temperature falls dramatically from 37ºC *in utero* to

approximately 25ºC in the delivery room. Because the newborn is coated with amniotic fluid, there is substantial evaporative heat loss. Circulating air currents in the delivery room contribute to convective heat loss. Conductive heat loss occurs when the infant is placed upon cooler surfaces such as unwarmed mattresses and blankets. Finally, heat is lost through radiation to cooler surfaces surrounding the infant but not in direct contact with the infant.

The newborn is capable of sensing this cold stress and immediately triggers homeothermic mechanisms to minimize further heat loss. Vasoconstriction of the dermal arterioles minimizes the flow of warm blood to the cooler surface of the skin. Non-shivering thermogenesis, in which brown fat stores undergo exothermic reactions thereby generating heat, is initiated. Despite these adaptive measures, the core temperature of the newborn will continue to fall unless intervention by caretakers occur in a timely manner.

The low birth weight infant is exquisitely susceptible to heat loss in the immediate postnatal period as they have a larger surface area to weight ratio and limited subcutaneous fat. The preterm infant, in particular, is less able to adapt to these environmental changes secondary to an immature epidermal barrier and a paucity of brown fat stores. For these reasons, the preterm infant has often been referred to as a functional poikilotherm.

It is generally accepted that the newborn should have a core body temperature at or near 37ºC. However, it is important to recognize that even a newborn with a normal core body temperature may be exerting a tremendous amount of energy to maintain this temperature and therefore, is cold stressed. While it has been recognized for decades that cold stress at birth has a negative impact upon infant survival, particularly in low birth weight infants [11], it is surprising to learn that despite dramatic advances in neonatal care, temperature management in the delivery room remains an ongoing challenge.

A large, multicenter, observational study published by Laptook et al [12] examined the distribution of body temperatures in low birth weight infants admitted to the neonatal intensive care unit. The study included more than 5,000 very low birth weight infants and found that nearly 47% had low admission temperatures defined as less than 36ºC. Futhermore, there was a significant association between the extent of reduced temperature upon admission and late-onset sepsis and in-hospital mortality. These findings were similar to those published earlier in the EPIcure study, where approximately 40% of infants less than 26 weeks gestation had admission temperatures below 35ºC and this was correlated with decreased survival [13].

Interventions to limit heat loss in the delivery room include the pre-warming of blankets and mattresses, rapidly drying the newborn and removing wet blankets from infant contact. Swaddling infants in a warmed blanket is generally sufficient for stable, term infants. Early initiation of kangaroo care, allowing for direct skin-to-skin contact with the parent, is another effective means of maintaining the newborn's temperature [14].

Overhead heaters are necessary for those infants requiring further resuscitative efforts. Occlusive wraps (polyurethane, polyethlylene, or polyvinyl) may be beneficial in minimizing heat loss in extremely preterm infants, as their use has been associated with higher admission temperatures to the intensive care unit [15].

While it has long been recognized that neonatal hypothermia is associated with decreased survival, potential morbidities related to neonatal hyperthermia have more recently been described [16].

Neonatal hyperthermia may occasionally be iatrogenic in nature (inappropriate use of radiant warmers, occlusive wraps). However, it most often results from an elevated maternal temperature secondary to epidural anesthesia, prolonged labor or chorioamnionitis. Shalak et al [17] examined a cohort of term infants with clinical chorioamnionitis and found that elevated temperature at 30 minutes of life was correlated with neonatal depression at birth (requiring positive pressure ventilation or Apgar score less than 5 at 5 minutes) and admission to the neonatal ICU [17]. Other investigators have demonstrated an association between maternal fever and neonatal encephalopathy [18]. Because these studies utilize maternal fever as a surrogate for neonatal hyperthermia, it remains unclear if maternal fever alone is sufficient to cause neurologic insult or if it is simply a marker for other intrapartum risk factors.

35.6 Assessment of Resuscitation Efforts: the Apgar Score

In 1952, Virginia Apgar created a scoring system to rapidly assess the physical condition of infants shortly after birth in order to recognize those infants in need of further interventions to establish breathing [4]. The Apgar score, which includes evaluation of heart rate, respiratory effort, muscle tone, reflex irritability and color, remains widely used today as a measure of the infants response to resuscitative efforts.

Several investigators have attempted to expand the utility of the Apgar score beyond the scope of the delivery room, using it as a measurement of risk for later impairment. While there is some evidence to suggest that a low 5 minute Apgar score may be associated with future neurologic disability, the American Academy of Pediatrics cautions against its use as a predictor of long-term outcome or as a specific marker of intrapartum asphyxia, as this was not the original intent [19]. Interestingly, a low 5 minute Apgar score (0–3) has been shown to be a valid predictor of neonatal mortality [20]. The Apgar score is affected by gestational age and therefore its significance in preterm infants remains unclear.

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36

Approach to Low Risk Newborns

Jennifer M. Duchon

36.1 Introduction

Of the approximately 4 million live births occurring annually in the United States [1] over 90% require nothing but routine care of the infant in the delivery room. The majority of these births are considered low risk, but the percentage of deliveries that are categorized as low risk has decreased in recent years. This is in part due to increasing numbers of births at the extremes of childbearing age, an increase in complications due to maternal morbidities such as obesity, and an increasing preterm birth rate.

In many cases, the need for newborn resuscitation can be anticipated. However, at every birth, the appropriate equipment should be available. Neonatal Resuscitation Program (NRP) guidelines and common sense recommend that at least one person skilled in neonatal resuscitation whose only responsibility is the management of the newborn is present at every delivery. This person does not need to be a physician, but should possess the ability to perform the initial steps in resuscitation until additional help arrives [2].

The initial steps in the management of the newborn will be discussed elsewhere in this text. Several key aspects of continuing care of the well newborn in the delivery room and during the recovery period will be discussed.

In the healthy term newborn the goal of delivery room care should be to assess the newborn for any features that may confer the need for closer monitoring and follow-up, and to promote the natural bonding between mother and infant. In this regard, much of the continuing care of the newborn can be done in such as way as to be minimally invasive to the infant and not disruptive to the mother-infant dyad.

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36.2 Routine Assessment

36.2.1 Physical Exam

Every newborn should be assessed with a brief physical examination in the delivery room. The complete physical exam of the newborn will be discussed in detail in later chapters, and has a different goal. As previously stated, the purpose of the physical exam in the delivery room should be to assess the newborn for any features that may indicate the need for immediate intervention or for further investigations and follow-up during the nursery stay. Important findings to identify are:

- visually patent and normally placed anus
- normal external genitalia
- presence of cleft lip or palate
- intact spine and sacral area.

Most major congenital malformations are discovered during routine prenatal care, and second trimester ultrasound at 18–22 weeks gestational age remains the most common imaging modality. When performed at tertiary care or University affiliated institutions, second trimester ultrasound has been shown to have good specificity and fair sensitivity for the detection of fetal anomalies [3]. However, defects such as those listed above, especially small facial anomalies, are frequently difficult to detect with traditional 2-dimensional ultrasound techniques. As more sophisticated techniques such as 3-dimenstional ultrasound or fetal magnetic resonance imaging (MRI) come into wider use, the detection rate for these anomalies, especially spinal and facial defects, may improve [4].

It is not recommended to perform a rectal probe exam, such as a rectal temperature, to document patency of the anus. Deep suctioning of the nasally or orally is also neither necessary nor recommended to determine patency of the nares or esophagus, as this may lead to trauma or perforation of the mucosal tissues. This is supported by recommendations from The American Academy of Pediatrics (AAP) section on breastfeeding, which states that unnecessary, excessive, and overvigorous suctioning of the oral cavity, esophagus, and

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airways may traumatize the infant and lead to aversive feeding behavior [5]. An early physical examination of the infant can also document normal variants such as molding or caput of the scalp, Mongolian spots or sucking blisters, and reassure parents of the benign nature of such findings. The initial physical assessment of the baby can be performed while skin-to-skin contact with the mother is taking place.

36.2.2 Thermal Regulation

Thermal regulation of the neonate, both preterm and full-term, is one of the major challenges faced in the delivery room. Heat loss in newborns occurs through four basic mechanisms: radiation, conduction, convection and evaporation.

Heat loss through radiation is related to the temperature of the surfaces surrounding the infant but not in direct contact with the infant, and is an important mechanism in heat loss in term infants. This is the rationale behind placing the infant on a radiant warmer immediately after delivery. However, the infant may emit radiant heat to a colder source, such as walls or windows. Conduction is the transfer of heat through direct contact with a surface that is colder or warmer, such as the bedding or swaddling that the infant is initially placed in. In convection, heat transfer occurs when air currents carry heat away from the body surface. The fetus generates heat, and the temperature of the newborn is approximately 0.5°C higher than the maternal temperature. Most delivery rooms are obviously colder than the newborn, and heat is first conducted into the air and then carried away by the convective air currents. This is an especially prominent phenomenon in the operating room, where the ambient air temperature is often kept cooler for the comfort of the garbed medical staff. Evaporation occurs when water is lost from the skin. During evaporation, approximately 0.6 kcal of heat is lost for every 1 g of water lost from the body [6–8]. Reducing heat loss though evaporation is a target of intervention that is especially important in the very preterm infant. In the full-term infant, the initial step of drying the infant with warm blankets ameliorates both evaporative and conductive heat loss.

Heat is generated by the newborn primarily though non-shivering thermogenesis. Non-shivering thermogenesis is the production of heat that does not occur through muscle activity. In neonates, this occurs primarily though the metabolism of brown fat. Brown fat, found in infants greater than 30 weeks, contains a high concentration blood vessels and sympathetic nerve fibers, and its metabolism causes an increase in sympathetic activity leading to an increase in norepinephrine, thyroid-stimulating hormone, T4 and T3. These mediators cause increased fat oxidation and heat production [6–9].

There are several ways to reduce heat loss and prevent resultant cold stress. The simplest method is that of skin-to-skin contact. The mother is an optimal heat source for the infant. Skin-to-skin contact has been well studied in many different cultures and resources settings, and has been shown to successfully maintain normal temperature of healthy term neonates. This type of contact can also alleviate mind hypothermia. It has been shown to promote bonding and facilitate both the initiation and continuation of breastfeeding [5, 6]. Other methods of preventing hypothermia in the newborn include placing a cap on the infant, and keeping the temperature of the delivery room such that heat loss due to evaporation and conduction, respectively, are minimized.

36.2.3 Initiation of Breastfeeding

The full physiology and nutrition of breastfeeding and breast milk will be discussed in Section III. For the purpose of the care of the infant in the delivery room, the initiation of breastfeeding may be considered one of the most important contributions to the health and well-being of a newborn. Briefly, the AAP recommendations for breastfeeding and breast milk include:

- breast milk for all infants for whom it is not medically contraindicated
- exclusive breastfeeding for the first 6 months of life
- continuation of breastfeeding for at least one year
- peripartum practices that facilitate breastfeeding.

The alert, healthy newborn infant is capable of latching on to a breast without specific assistance within the first hour after birth [5, 10]. In keeping with the previous discussion on temperature regulation, the AAP recommendations to promote breastfeeding in the delivery room encourage that well, full-term infants be placed and remain in direct skin-to-skin contact with their mothers immediately after delivery until the first feeding is accomplished [5, 10]. Weighing, measuring, bathing, and other non urgent interventions should be delayed until after the first feed is completed. For both bonding and breast feeding purposes, the newborn infant should remain with the mother throughout the recovery period. A Cochrane review confirms that institutional changes in maternity care practices effectively increased rates of breastfeeding initiation and duration [11]. Institutional policies surrounding routine care of healthy newborn should be developed with the goal of safety and the promotion of the mother infant bond, as opposed to convenience of the medical and ancillary staff.

36.3 Vital Signs and Measures

36.3.1 Vital Signs

Non-invasive vital signs, such as heart rate, respiratory rate and temperature, as well as the procedure of weighing and measuring the infant, are often performed in the delivery room. Some institutions also have protocols involving more

invasive measures, such as screening for oxygen saturation, hypoglycemia, anemia or polycythemia.

A vigorous term infant who has been ascribed good APGARS is unlikely to have major derangements in pulse or respiratory rate. Mild tachypnea may be a physiologic part of the transition from fetal to extrauterine life. The increased pulmonary vascular resistance of the newborn as manifest by oxygen saturation takes time to normalize. Studies have demonstrated that healthy full-term neonates rarely reach oxygen saturations of greater than 90% until after the first 10 minutes of life, and may have lower post-ductal saturations for an even longer period [12, 13].

36.3.2 Hemoglobin and Hematocrit

Healthy full-term infants have, in past years, been targeted for treatment of anemia or polycythemia based on values obtained from routine screening at birth. This can be troublesome from several perspectives. The definition of both anemia and polycythemia can vary. Most practitioners define anemia as a greater than 2 standard deviations below the mean hemoglobin, or less than the fifth percentile. For full-term neonates, this is approximately 13 g/dL from a sample of blood drawn centrally, or 14.5 g/dL drawn from capillary sampling. Symptoms of anemia in the immediate newborn period may range from pallor to tachypnea to severe respiratory distress and circulatory collapse. Polycythemia can be defined in a similar manner, but has traditionally been diagnosed as a hematocrit greater than 65% drawn centrally or 70% drawn from a capillary specimen. Risk factors include macrosomic infants, or infants who are born to mothers who have conditions that affect placental blood flow, as a compensatory mechanism from the fetus.

Symptoms of polycythemia include central nervous system (CNS) manifestations such as lethargy and tremulousness, hypoglycemia, as well as evidence of organ failure such as respiratory distress, renal failure, congestive heart failure, or intestinal symptoms. Both obstetric practices, such as method of delivery, timing of cord clamping and positioning of the infant relative to cord clamping, can affect these values, as do the timing of blood draw relative to delivery [14, 15]. The degree to which an individual infant is symptomatic at a particular hemoglobin or hematocrit is extremely variable. It is also very difficult to differentiate the clinical effects, both short and long-term, of polycythemia and anemia in an asymptomatic infant, from the effects of the cause of the abnormal indices. Especially for polycythemia, there is no evidence to suggest that the treatment modality, partial exchange transfusion, of an asymptomatic infant improves outcome. The most sensible approach would be to consider screening infants who are at risk for anemia from fetal or intrapartum complications, and may warrant closer follow-up, or for any infant who displays symptoms of abnormal hemoglobin or hematocrit [15].

36.3.3 Monitoring of Glucose

There has been much discussion in recent years as to the measurement and management of glucose levels in the low risk infant.

The fetus has a complete and continuous supply of glucose from the mother from placental transfer, via carrier mediated facilitated diffusion. After birth, when this supply is abruptly cut off, there are several mechanisms to maintain adequate energy to the infant:

- glycogenolysis
- gluconeogenesis
- lipolysis
- fatty acid oxidation and ketogenesis
- hormonal/endocrine regulation of these systems.

Serum glucagon, catecholamines and growth hormone rise after cord clamping and insulin levels fall, favoring glycogenolysis, lipolysis and gluconeogenesis. Full-term infants are born with adequate glycogen stores, but these are depleted within the first several hours after birth unless feeding is established. Lipolysis can occur shortly after birth, and releases free fatty acids that can be used as an energy source by many tissues, although not by the brain. Fatty acid oxidation and ketogenesis take place in the liver, and produces ß-hydroxybutyrate, acetoacetate and ketones which can be used by as an energy source for the brain. Several of the major hormones required for control of these systems rise in the first few hours of life, leaving the production of usable energy from both gluconeogenesis and hepatic ketogenesis delayed. This leaves free fatty acids from lipolysis and glucose from breakdown of glycogen stores as the major energy sources immediately after birth [8, 16]. In infants with poor stores of adipose tissue or glycogen, such as preterm or growth restricted infants, maintaining an adequate supply of fuel for the metabolic needs of the brain becomes of great concern. Infants who are well may also be at risk. Non-shivering thermogenesis is the major mechanism of heat production in the neonate, and cold stress in the delivery room can occur. A newborn infant left unattended in an environment at typical "room temperature" experiences energy losses of approximately 150 kcal per minute, rapidly using up energy stores [6]. Delay in enteral feeding for routine newborn care can also exacerbate hypoglycemia. This provides further evidence for care to be performed skin–to-skin with the mother with early initiation of breastfeeding.

Many nurseries still monitor serum glucose levels in low risk, healthy infants as part of routine care. A problem can then arise, as debate occurs about what level of serum glucose should be considered abnormal and require intervention in an asymptomatic, otherwise well newborn. When it was first recognized as a pathologic state, hypoglycemia was defined as less than 20 mg/dL (1.1 mmol/L) in preterm infants and less than 30 mg/dL (1.7 mmol/L) in full-term infant. Cohort studies later showed that infants with symptomatic hypoglycemia had poorer neurodevelopmental outcomes [17]. Since that time,

there has been little agreement about minimum acceptable level of glucose in healthy term infants. Some authors contend that 45 mg/dL (2.5 mmol/L) is the lower limit for all infants [18, 19]. Other maintain that due to the key time in development, and lack of evidence for compensatory mechanisms that protect the neonatal brain from hypoglycemic injury, glucose values should be the similar in newborns as in older children, greater than 60 mg/dL or 3.3 mmol/L [16]. Symptoms from hypoglycemia include tremulousness, lethargy, seizures, hypothermia, or can mimic respiratory distress. Infants who are considered at risk for hypoglycemia are those who are small or large for gestational age, or who have maternal risk factors for abnormal glucose such as gestational diabetes or medications affecting glucose metabolism. Also at high risk for sequelae from hypoglycemia are those infants who have evidence of infection, hypothermia, polycythemia or hypoxia-ischemia [18, 19]. It is generally accepted that healthy term infants, either breast-fed or formula-fed, who do not have risk factors for compromised metabolic adaptation and who are asymptomatic need not have routine glucose monitoring [15]. Any infant who appears symptomatic from hypoglycemia, or in whom risk factors for increased altered glucose metabolism exist should be evaluated and treated as necessary.

36.4 Common Routine Treatments

36.4.1 Eye Care

Neonatal ophthalmia is defined as conjunctivitis that occurs within the first 28 days of life. It is a relatively common illness, occurring in 1–12% of newborn infants. Originally, neonatal ophthalmia referred to conjunctivitis in the newborn caused by infection with *Neisseria gonorrhoeae*, but now the term refers to any conjunctivitis in this age group, regardless of the cause [20]. Gonococcal ophthalmia was, in the past, a leading cause of blindness, but is now rare in most developed populations with access to treatment and screening during pregnancy. It is for this historical reason that delivery room prophylaxis was geared towards prevention of blindness from this pathogen. Since the diminishment of gonococcal ophthalmia, there has been debate about which agent, if any, to use as prophylaxis against conjunctivitis in the delivery room. Most cases of conjunctivitis in the neonatal period are due either to chemical conjunctivitis or non sexually transmitted colonizing bacteria. *Chlamydia trachomatis* also causes a portion of ophthalmia, depending on local incidence. With the exception of gonococcal and chlamydial conjunctivitis, the condition is mild, and usually responds well to local treatment with no long-term sequelae with appropriate therapy. It is the consensus from the AAP, as well as the Canadian Paediatric Society (CPS) that eye prophylaxis, in the form of 1% silver nitrate, 0.5% erythromycin, or 1% tetracycline drops or ointment be given. The medication should be instilled as soon as possible in the delivery room, and should not be wiped off. There is no data suggesting that delaying administration until after the first breastfeed in any way affects efficacy. Erythromycin ointment has replaced other forms of prophylaxis in the US, as some initial data suggested that there may be protection against chlamydial species. Later studies disproved this [21] probably due to the fact that local treatment will not eradicate nasophargyngeal colonization of the organism. Many US physicians feel that erythromycin causes less chemical conjunctivitis than silver nitrate and the drug remains in wide usage. This is not the case in other countries, where aminoglycoside, chloramphenicol, or no prophylaxis is used. As demonstrated by Guala et al [22], agents used as prophylaxis against neonatal ophthalmia vary widely between centers and are functions of local practice and tradition, as opposed strictly to evidence based medicine.

36.4.2 Vitamin K

Due to limited stores at birth, neonates are prone to vitamin K deficiency if no sufficient intake is provided. The clinical syndrome associated with vitamin K deficiency has been termed hemorrhagic disease of the newborn, and comprises three distinct presentations. The very early form presents within 24 h of birth and is almost exclusively seen in infants of mothers taking drugs which inhibit vitamin K. These drugs include certain anticonvulsants, some antibiotics and vitamin K antagonists, many of which are now avoided in pregnancy. Clinical presentation can be severe, with cephalohematomas, intracranial and intra-abdominal hemorrhage. Classical vitamin K deficiency occurs between 24 h and 7 days of life and is associated with delayed or insufficient feeding. Clinical presentation is often mild, with bruises, gastrointestinal bleeding, or bleeding from the umbilicus and puncture sites. The late presentation of vitamin K deficiency is associated with exclusive breast-feeding. It occurs between the ages of 2 and 12 weeks and infants can be gravely ill, with a mortality rate of 20%. Intracranial hemorrhage occurs in up to 50% of those affected [23]. After the discovery of vitamin K in the mid 20th century, it was shown that treatment with vitamin K could abolish hemorrhagic disease of the newborn. It then became standard practice to administer the drug soon after birth to all infants. Controversy has arisen about this practice for several reasons. Both classic and late vitamin K deficiency are relatively rare: recent reviews cite estimates of 0.01–0.44% in the general population for the classic form, and between 4.4/100,000 and 7.2/100,000 births for the late form in fully breast-fed infants who did not receive vitamin K at birth [23]. The present dose of 1 mg represents a very large amount when compared to the daily requirement of 5–10 μg in infants. The dose of 1 mg appears to have been chosen fairly arbitrarily, with no formal studies being performed to establish what dose

might be appropriate. Intramuscular form became the route of choice based primary on the available formulation at the time [24]. In the 1990s, a study from Britain linked the administration of intramuscular vitamin K at birth to childhood cancers and leukemia [25]. Later studies failed to confirm this association, but the guidelines for universal prophylactic vitamin K were revised in many countries to include enteral dosage. It is universally accepted that vitamin K is necessary to prevent all forms of clinical disease in newborns. However, the method of administration and timing remain non-uniform across guidelines. For infants at risk from maternal medications, traumatic delivery or prematurity, the intramuscular route is preferred. For healthy term newborns at low risk, the CPS recommends that vitamin K should be given as a single intramuscular dose of 1.0 mg to all newborns within the first 6 hours after birth. For infants whose parents refuse an intramuscular injection, an oral dose of 2.0 mg of vitamin K at the time of the first feeding with dosages repeated at 2–4 weeks and 6–8 weeks of age may be used [26]. Some countries have investigated the use of a small daily dose of enteral vitamin K in lieu of large doses less frequently in breastfeeding infants who did not receive the injection at birth, with apparent success [23]. Parents choosing oral dosing should be advised of the necessity of follow-up doses and be cautioned that their infants remain at an increased risk of late vitamin K deficiency (including the potential for intracranial hemorrhage) using the oral as opposed to parenteral route [26].

36.5 Conclusions

A 1995 study performed across several Italian nurseries showed that despite the availability of evidence based national and international guidelines pertaining to routine newborn care, practices were guided by long standing habit and previously developed experiences [22]. This can be extrapolated to many aspects of neonatal care. However, in the low risk, well newborn, the guiding principle should be to ensure the health and well-being of the baby, and facilitate practices that will encourage continued health through maternal child interaction.

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37

Early Detection of Neonatal Depression and Asphyxia

Paolo Biban and Davide Silvagni

37.1 Introduction

Perinatal asphyxia is an insult to the fetus or newborn due to hypoxia and/or ischemia, persisting long enough to cause pathological biochemical changes and a variable degree of injury to various organs, including the brain. The effects of hypoxia and ischemia are often difficult to separate clinically. Hypoxia refers to an arterial concentration of oxygen that is less than normal, while ischemia occurs when the blood flow to the cells or organs is insufficient to maintain normal function. In its more severe forms, the impaired gas exchange secondary to asphyxia is also associated with tissue lactic acidosis and hypercapnia [1].

Neonatal depression is a general term to describe the condition of any newborn in the immediate postnatal period (approximately the first hour after birth) showing a prolonged transition from intrauterine to extrauterine life. The degree of depression is described by the Apgar score: a 1 minute score of 4–6 is considered as moderate depression, and a score of 0–3 indicates severe neonatal depression, requiring the institution of immediate resuscitation.

37.2 Incidence and Definition of Neonatal Asphyxia

Despite major advances in monitoring technology, obstetric care and knowledge of fetal and neonatal pathologies, asphyxia remains a serious condition causing significant mortality and long-term morbidity. According to the World Health Organisation (WHO), 4–9 million cases of newborn asphyxia occur each year [2]. More than a million newborns that survive asphyxia at birth develop long-term problems such as cerebral palsy, mental retardation, speaking, hearing, visual and learning disabilities [2]. Globally, it is estimated that out of 4 million neonatal deaths annually, nearly one fourth are attributable to asphyxia [3]. In the developing world the incidence of asphyxia is believed to be considerably higher due to the increased prevalence of risk factors. However, the incidence of birth asphyxia may vary markedly, depending on the definition used, as well as the gestational age of the infant.

Many definitions of perinatal asphyxia can be found throughout the available literature, but a universal definition is still lacking [4–6]. One of the most utilized is that promulgated in 2003 by the American Academy of Pediatrics and the American College of Obstetrician and Gynecologists, which includes the following criteria [7]:

1. metabolic or mixed acidosis (pH < 7.00) in an arterial cord blood sample;
2. Apgar score 0-3 for more than 5 minutes after birth;
3. evidence of neurologic signs in the immediate neonatal period;
4. evidence of multiorgan failure in the immediate neonatal period.

Newborn infants suffering episodes of perinatal asphyxia are at high risk of dying or developing brain damage. Postasphyxial hypoxic-ischemic encephalopathy (HIE) is viewed as the hallmark and most important consequence of asphyxia, being a major cause of death and of disability in term and near-term newborn infants in developed countries [8].

Approximately 1–2 infants per 1000 live term births are affected by hypoxic-ischemic encephalopathy, with outcomes ranging from complete recovery to death [9]. HIE is a well-recognized clinical syndrome, with a large spectrum of clinical manifestations ranging from mild to severe. The clinical staging of Sarnat and Sarnat has been widely used since the 1970s to estimate the severity of the hypoxic-ischemic insult in infants of 36 or more weeks of gestation [10]. In most cases, the severity of the encephalopathy predicts the risk of death and long-term neurodisability [10, 11]. However, an hypoxic ischemic encephalopathy secondary to intrapartum asphyxia cannot reliably predict the consequent neurodevelopmental

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outcome [8, 12]. Most term newborns with perinatal asphyxia have uneventful courses and a low likelihood of subsequent neurological sequelae [8, 13]. Perinatal depression, when defined as low Apgar scores in the first minutes of life, may be not only related to birth asphyxia but also to other factors carrying a much lower risk of unfavorable short and long-term outcome, including a vagal response due to nasopharyngeal stimulation, low gestational age, depression because of maternal anesthesia [8]. The prognosis for any given baby remains uncertain and reliable diagnostic and prognostic indicators related to perinatal asphyxia are still needed. Accurate markers of severe asphyxia and predictors of outcome would facilitate parental counselling as well as the choice of appropriate levels of care, which may include withdrawal of intensive care or the initiation of neuroprotective strategies.

Perinatal asphyxia may be categorized as occurring in three phases: 1) intrapartum; 2) immediate postpartum and 3) early neonatal period.

37.3 Intrapartum Asphyxial Markers

Assessment of the intrapartum condition of the fetus includes electronic fetal heart rate monitoring (EFM), the observation of meconium in the amniotic fluid, fetal scalp blood sampling for acid-base and lactate measurements.

Electronic fetal monitoring Electronic fetal monitoring (EFM) has been used to predict fetal distress or well-being. Heart rate abnormalities have been identified as a marker of asphyxia during labor, fetal bradycardia being one of the most serious [14]. However, EFM has been shown to be imprecise in detecting fetuses with metabolic acidosis and predicting those likely to develop an hypoxic-ischemic encephalopathy [15, 16]. Furthermore, in low-risk pregnancies intrapartum EFM has led to an increase in cesarean and operative vaginal delivery rates, but not to a documented improvement in neonatal outcome. Newer technolo- gies, such as fetal pulse oximetry and fetal electrocardiography may more accurately identify fetuses at risk. Other techniques, such as Doppler measurement of blood flow velocity, near infrared spectroscopy and fetal electroencephalogram, although useful are less suitable for a routine clinical application [8].

Meconium passage in utero Meconium passage in utero is frequently cited as a sign of fetal stress, but several studies have failed to demonstrate a correlation between meconium stained amniotic fluid and permanent neurological sequelae [17]. Currently, meconium passage in utero is considered neither a significant nor a specific marker of fetal distress unless associated with other abnormalities [8, 18].

Fetal scalp blood sampling Fetal scalp blood sampling (FBS) was introduced by Bretscher and Saling in 1962 and was primarily based on pH analysis [19]. They defined pH cut-off values as normal (>7.25), pre-acidotic (7.20–7.25) and acidotic (<7.20), recommending prompt delivery for cases with acidosis. These guidelines are still regarded as the "gold standard" for diagnosing intra-partum fetal distress. Fetal acidemia due to an hypoxic-ischemic insult is usually metabolic and a consequence of anaerobic glycolysis due to prolonged intrauterine hypoxia. In a large retrospective study, Kruger et al [20] performed 814 lactate and/or 1221 pH scalp blood measurements when fetal distress was suspected. In terms of sensitivity and specificity, lactate performed better than pH in the prediction of severe neonatal morbidity [20]. In addition, Kruger et al [21] demonstrated a good correlation between lactate obtained by FBS close to delivery and from cord arterial blood immediately after delivery. The potential usefulness of fetal scalp and cord blood lactate measurements to monitor the peripartum conditions of the fetus has been recently reviewed by Nordstrom [22].

Fetal scalp blood acid-base and lactate measurements, especially when combined with fetal heart rate monitoring, seem to be of value in predicting the condition of newborns at birth. However, this invasive approach can be performed only after rupture of membranes and is not widely used. Associated complications, although rarely observed, are soft tissue damage and scalp infections [23].

37.4 Immediate Postpartum Asphyxial Markers

Common markers of asphyxia used immediately after birth include low Apgar scores, umbilical artery acidemia and high blood lactate concentrations.

Apgar score The Apgar score was originally used as a rapid method of assessing the need for newborn resuscitation and to define newborn asphyxia [24]. However, the Apgar score has a number of limitations, mainly due to a considerable inter-observer variability and the different physiological implications of the five factors which make up the final score. Furthermore, causes other than asphyxia may result in low Apgar scores but without a high risk of brain injury, such as resuscitation procedures, infections, congenital malformations, maternal anesthesia [7]. Moreover, Apgar scores assigned during mechanical ventilation are not equivalent to scores assigned to spontaneously breathing infants, and the significance of low Apgar scores calculated in extremely premature babies are likely to differ from those awarded to term or near-term infants.

Finally, although the Apgar score has been used to predict specific neurological outcome in the term infant, several studies have demonstrated a weak correlation between low Apgar scores and severe birth asphyxia and subsequent adverse neurological development [13]. Nevertheless, the Apgar score is widely used and is useful for describing the condition of newly born infants and their response to resuscitation. It is

not appropriate to use it alone to establish the diagnosis of asphyxia, but, when combined with other conventional markers, such as arterial umbilical cord pH and base deficits, it has some predictive value for the development of hypoxic-ischemic encephalopathy [20, 25].

Acid-base measurements in umbilical cord blood Because Apgar scores are poor markers of severe birth asphyxia, some investigators have suggested the use of early acid-base status as a more objective indicator and the finding of a severe metabolic acidosis in the umbilical cord arterial blood at birth is probably the most objective assessment of intrapartum hypoxic ischemia [7]. Metabolic acidosis occurs because of a prolonged tissue oxygen deficit, reflecting hypoxia or impaired blood gas exchange of significant duration to allow the accumulation of fixed acids, mainly lactic acid. Most studies assess acid-base status on the arterial cord blood as soon as possible after birth [7]. The acid–base criteria commonly used to diagnose intrapartum asphyxia are a pH <7.00 and a base deficit (BD) value >16 mmol/L in umbilical arterial blood at birth or during the first postnatal hour [5, 7, 26].

In a cohort of term infants with an umbilical arterial pH <7.00, nearly two-thirds did not require admission in NICU and had no apparent neurological sequelae [27]. The need for cardiopulmonary resuscitation and adrenaline administration in the delivery room identified those neonates with pH <7.00 at greatest risk for a short-term adverse outcome [27]. In a comprehensive review, Graham et al [16] searched for studies reporting the incidence of umbilical arterial pH <7.00. Combining data from seven different studies, they found the mean incidence of umbilical arterial pH <7.00 at term in 3.7/1000 infants. Of 386 infants, about 17% survived with neurological morbidity, 16% developed seizures, and 6% died during the neonatal period. The combined incidence of neonatal neurological morbidity and mortality for term infants with an umbilical arterial pH <7.00 at birth was 23%, with the remaining 77% being neurologically normal at the time of neonatal discharge [16]. In another study, the incidence of encephalopathy in 109 term infants with umbilical artery pH <7.00 was 31%: seizures occurred in 9% of infants with pH between 6.90 and 6.99, and in 80% of those with pH between 6.61 and 6.70 [28]. In a smaller study, Nagel et al [29] reviewed 21 infants born with a pH <7.00 over a 19-month period. Two out of 21 infants died in the neonatal period, but when the survivors were evaluated at 1–3 years, they showed normal developmental scores. Therefore, even though a pH value <7.00 at delivery seems to be a good marker of severity of acidosis, it appears less well related to short- and long-term neurological outcomes.

Conventionally, in the newborn the degree of metabolic acidosis is measured also by the umbilical artery base deficit. Studies have shown that a base deficit excess greater than 16 mmol/L is predictive of neonatal encephalopathy. Low et al [30] observed a significant increase in moderate and severe encephalopathy with an umbilical artery base deficit >16 mmol/L, suggesting that a base deficit excess measured in arterial cord blood is a good index of prolonged asphyxia, reflecting the severity and duration of intra-partum asphyxia.

Recently, Shah et al [31] evaluated the rate of recovery of base deficit in 244 term infants with HIE due to intra-partum asphyxia. In their series, BD values normalized by the first 4 hours in the 96% of infants. Notably, the BD recovery rate of infants with severe adverse outcome at 12 months was similar to those with a relatively good outcome. Thus, although the rate of recovery of base deficit may reflect the ability of the infant to recuperate from the oxygen debt, it is not predictive of long-term outcome [31].

Blood lactate at birth Lactate is produced as a consequence of hypoxia and poor tissue perfusion [32]. When a critical reduction in oxygen and substrate delivery occurs, aerobic metabolism through Kreb's cycle cannot be sustained and tissues use anaerobic metabolism to meet their energy requirements. This in turn leads to an increase in the production and accumulation of blood lactate [33]. A significant association has been found between blood lactate concentrations and neurological evolution. da Silva et al [32] measured blood lactate at 30 minutes of life in 115 term infants with suspected asphyxia. Significantly higher blood lactate concentrations were observed in neonates with moderate to severe HIE [32]. In a group of 61 full-term infants with suspected asphyxia, Shah et al [34] observed that blood lactate concentrations were significantly higher and took longer to normalize in neonates with moderate to severe HIE, compared to those with mild or no HIE. Furthermore, they reported a higher risk of neurological and systemic complications when the blood blood lactate concentrations were more than 7.5 mmol/L at 1 hour of age with a sensitivity of 94% and a specificity of 67%. In their study the sensitivity and negative predictive value of lactate in the first hour of life were greater than either pH or base deficit [34]. A recent study by Murray et al [35] found that a normalization time of blood lactate concentrations greater than 10 hours was associated with a high risk of encephalopathy.

37.5 Asphyxial Markers in the Early Neonatal Period

Suspected intrapartum asphyxia causing brain injury can be confirmed in the first hours after birth by various clinical, biochemical and other measures. Some authors have attempted to develop models able to predict early and reliably the development of HIE and/or subsequent neurological sequelae in asphyxiated newborns. In a large retrospective cohort study, Shah et al [36] tried to validate a prognostic model for term infants with moderate to severe postasphyxial HIE. They used relatively simple clinical and laboratory observations available within the first 4 hours of life, and identified three significant

predictors: chest compressions >1 minute, onset of spontaneous breathing >30 minutes and a base deficit value >16 mmol/L in any blood gas analysis within the first 4 hours. In this cohort of term infants with HIE, a severe adverse outcome was found in 93% of subjects in whom all three predictors were present. However, infants with none of the three predictors also had a high possibility of adverse outcome (46%) [36]. More accurate prediction models are therefore still needed for the early identification of those infants most likely to benefit from interventions. At present, the occurrence of abnormal neurological features after signs of intrauterine asphyxia, namely postasphyxial hypoxic-ischemic encephalopathy, remains the best indicator of a newborn infant at risk of subsequent neurological impairment [8]. Other early markers include multiorgan dysfunction, urinary lactate/creatinine ratio and nucleated red blood cells counts. Other useful techniques include early electrophysiological and neuroimaging studies, such as amplitude integrated electroencephalogram (aEEG), cranial ultrasound, magnetic resonance imaging (MRI) and computed tomography (CT) (see also Chapter 132).

Postasphyxial neonatal encephalopathy Neonatal encephalopathy is characterized by abnormal consciousness, tone and reflexes, and seizure activity. The main cause of neonatal encephalopathy is intrapartum hypoxic ischemia, followed by trauma, infections, metabolic diseases and developmental abnormalities. Prognosis is related to the severity of the HIE syndrome, the onset of seizures and the duration of the neurological abnormalities.

Although several classifications are available, the most popular remains that proposed by Sarnat et Sarnat in 1976 to indicate the severity of the hypoxic ischemic insult in infants of 36 or more weeks of gestation [10]. The sequential appearance and resolution of the various transient clinical signs in the first 2 weeks suggest the extent of neurologic impairment and define different clinical categories in the early assessment of infants with HIE [1]. The Sarnat and Sarnat staging of HIE ranges from mild (grade I) to severe (grade III). Grade I comprises a mild encephalopathy with the infant being hyper alert, irritable and oversensitive to stimuli, but with a normal EEG. Virtually all these patients have a normal neurological outcome. In grade II there is a moderate encephalopathy, with the infant displaying lethargy, hypotonia and proximal weakness, low resting heart rate, small pupils. The EEG is abnormal and 70% of the infants will have seizures. About 20% of these patients may manifest abnormal neurological outcome. In grade III, there is a severe encephalopathy with the infant stuporous, flaccid and no reflexes. EEG is abnormal with decreased background activity and/or voltage suppression. About half of these patients die, whereas the surviving 50% suffer major neurological injury, with epilepsy and mental retardation [1, 10].

Multiorgan failure The American College of Obstetrician and Gynaecologists considered multiorgan failure (MOF) as a constant feature of the neonatal post-asphyxial syndrome [37]. However, this assumption has been questioned by several authors and the incidence of MOF in asphyxiated infants is variable. Such variability may partly depend on differences of selection criteria, definitions of MOF, or timing of the diagnosis of MOF.

In term newborns with arterial cord pH <7.00, Goodwin et al [28] observed an incidence of MOF of 30%. In a retrospective cohort study, all infants with severe HIE had evidence of MOF. However, the presence of MOF, regardless of the combination of different organs involved, did not correlate with long-term neurological outcome [38].

Thus, MOF may reinforce the suspicion of intrapartum hypoxic ischemia in newborns with HIE, but it is not a useful criterion for the early identification of infants at risk for an adverse neurological outcome.

Urinary lactate/creatinine Huang and coworkers investigated the role of urinary lactate/ creatinine ratio to differentiate normal infants (58 subjects) from infants with asphyxia (40 subjects) [39]. The measurements of urinary lactate and creatinine were obtained by proton nuclear magnetic resonance spectroscopy (1H NMR) within 6 hours of birth and at 48 and 72 hours. Sixteen of the infants with asphyxia went on to develop an encephalopathy. Within 6 hours after birth, the mean lactate/creatinine ratio in infants who subsequently developed encephalopathy was 186 times as high as the ratio in normal controls, and 88 times as high as that in infants with asphyxia not progressing to HIE. A urinary lactate/creatinine ratio of 0.64 or higher showed 94 percent sensitivity and 100% specificity for predicting the development of HIE. Thus, even if measurement technique by 1H NMR is expensive and not readily available, urinary lactate/ creatinine ratio could be a potentially useful marker for the early identification and treatment of infants at high risk for HIE [39]. Confirmation of these preliminary results is still needed.

Nucleated red blood cells The nucleated red blood cell (NRBC) count is a marker of fetal hypoxia in term and preterm infants. In 1996, Korst et al [40] reported a raised NRBC in cord blood from a group of neurologically impaired term infants compared with controls. In a prospective study, Buonocore et al [41] observed that the NRBC count in umbilical cord blood was significantly higher in infants showing abnormal neurodevelopment at age 3 when compared to normal controls.

Amplitude integrated EEG Continuous monitoring by amplitude integrated EEG (aEEG) can be done soon after admission to a NICU. It is helpful in the early assessment of the asphyxiated term newborn [42]. Changes in background activity and seizure activity are relatively easy to identify, allowing for prompt intervention. aEEG may provide useful information about the functional integrity of the brain, presence or onset of subclinical seizure activity and the effect of anticonvulsant drugs. It may also aid the selection of patients

suitable for neuroprotective interventions and in the early prediction of neurodevelopmental outcome [43–45]. Some authors have shown that aEEG can predict outcome accurately at 6 hours of age following intrapartum hypoxic ischemia with a positive predictive value of 84–86% and a negative predictive value of 92–96% [44, 45].

Early neuroimaging investigations Advanced neuroimaging techniques such as cranial ultrasonography (CUS), MRI and CT can be used in the early post-partum phase. CUS is a non invasive and widely available technique. Up to 50% of newborns with HIE may initially show a normal CUS. Furthermore, it is not difficult to visualize parasagittal cerebral lesions, cortical or brain stem injuries.

CUS is of value in the identification of focal areas of necrosis (especially if hemorrhagic) in the thalamus and basal ganglia as well as for the detection of necrotic and cystic components of periventricular white matter injury (PVL) and, although not optimal, in the evaluation of focal ischemic injuries. CUS may also be used to study the evolution of brain lesions [8].

MRI is considered the gold standard for the early evaluation of the infant with hypoxic-ischemic injury. Particularly with the development of diffusion weighted imaging, MRI is considered by some to be the method of choice to assess the newborn brain even in the first few days of life, providing valuable information on the location and extent of the neurological damage in moderately to severely asphyxiated infants [46, 47].

Because of radiation exposure and its poorer imaging definition compared to MRI, CT is less frequently used for infants with hypoxic-ischemic injury. CT is, however, still occasionally useful for the identification of focal ischemic or hemorrhagic brain injuries and when MRI is not readily available [8].

New biochemical markers in early detection of neonatal asphyxia Newer early markers of perinatal asphyxia such as S-100 Protein and activin A have been recently investigated [48, 49]. Activin A, a glycoprotein expressed in the central nervous system, has been found in higher concentrations in newborns with perinatal hypoxia and in infants developing HIE. In a recent study, Florio et al [49] measured activin A in urine collected immediately after birth in 30 severely asphyxiated term newborns and 30 healthy controls. Urine activin A was significantly higher in patients with moderate or severe HIE compared to controls. Activin A > 0.08 μg/L had a sensitivity of 83% and a specificity of 100% for predicting the development of moderate or severe HIE. The sensitivity and specificity increased to 100% and 98% between 12 and 72 h, respectively [49].

37.6 Conclusions

Early detection of perinatal asphyxia still constitutes a challenging target in neonatology. In addition, birth asphyxia can be associated either with an uneventful course or with significant mortality and long-term morbidity. Therefore, reliable diagnostic and prognostic indicators are essential for a prompt recognition and treatment of newborns at risk for subsequent negative outcome.

In the last decades many biochemical markers of asphyxia have been identified, both in cord blood and in blood samples after birth, including arterial pH, base excess and lactate. However, such indicators have shown a limited value in predicting long-term neurological outcome.

Other promising biochemical markers, e.g. S100 B protein, Activin A, urinary lactate/creatinine ratio or nucleated red blood cells, still need further evaluation before their inclusion in the routine assessment of asphyxia.

Some recent electrophysiological and neuroimaging investigations, such as amplitude integrated EEG and diffusion weighted MRI, may also provide early valuable information on brain damage and outcome of asphyxiated babies. However, these techniques are not always available and could be difficult to perform in critically ill neonates.

Further research is still required to overcome the limitations due to the assessment of perinatal asphyxia by single parameters. The combination of clinical and biochemical indicators should aim to validate prediction models capable to identify neonates at risk of dismal outcome due to asphyxia, allowing an adequate and timely diagnosis, treatment and counselling.

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38

Resuscitation of the Newborn

Ola D. Saugstad

38.1 Introduction

Each year 6–10 million of the approximately 130 million newborn infants born in the world need some kind of resuscitation at birth. In the USA 5–10% of all newborn infants require basic life support in the delivery room, or nursery, constituting at least 200,000 newborn infants in the USA alone. In Western Europe there are similar figures. Approximately 1% of newborns require more extensive resuscitation procedures [1–3]. Recent estimates indicate that worldwide 814,000 die and an equal number develop sequels after birth asphyxia [4].

However, data from for instance California indicate that birth asphyxia is decreasing from 14.8 to 1.3 per 1000 in the period of 1991-2000 and data from England show that there is a reduced need of ventilation at birth [5, 6]. In spite of that there is no doubt that, both in industrialised and low income countries a large number of newborn infants are in need of resuscitation at birth. Therefore and because a vast number of deliveries throughout the world still occur at home without authorized health personnel present, it is also important to develop simple resuscitation routines.

38.2 Preparation for Resuscitation and Selection of Risk Cases

Approximately 95% of deliveries are uneventful and the adaptation to extrauterine life occurs smoothly. Only in some infants should more vigorous resuscitation procedures be carried out. As mentioned above, as many as 5% or more need basic resuscitation including ventilation using a bag and mask. However, only a few newborn infants need advanced resuscitation. In 1:100 to 1:700 endotracheal intubation is needed, 1:1000 needs chest compression, 6:10,000 need epinephrine (adrenaline) and as few as 1:12,000 of near-term and term infants need volume therapy [6–8]. For preterm infants these numbers are higher.

It is important to anticipate which infants are at risk and the antepartum and intrapartum history often can be a help in predicting which infants will need resuscitation. Fetal-movement counting, non-stress testing, fetal biophysical profile, electronic fetal monitoring, and scalp pH measurement all contribute to an identification of infants at risk for low Apgar scores and need of resuscitation. Electronic fetal-heart monitoring combined with scalp pH determinations improves the specificity; however, sensitivity is low. In spite of these measures, the need for resuscitation can often not be foreseen and 20-50% of the infants requiring resuscitation have no identifiable risk factors. This implies that at every delivery the health provider should be prepared to perform resuscitation. Equipment and facilities should be ready. Further, every health personnel attending deliveries need to be trained in resuscitation skills.

38.3 Indication for Resuscitation

The decision to resuscitate is based on clinical judgement. By observing breathing movements, tone, colour, and not least heart rate, it is decided whether the infant is in need of resuscitation or not. Every time a child is delivered the following three questions should according to the International Liaison Committee on Resuscitation (ILCOR) guidelines [1] be answered: 1) Is the baby breathing or crying? 2) Is there a good muscle tone? 3) Is the baby born at term? If the answer is "no" to any of these questions resuscitation should be considered. In former guidelines [9, 10] a fourth and a fifth question were included: 4) Is the colour pink? 5) Is amniotic fluid clear of meconium? Recent data, however, indicate that a

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

newborn baby not necessarily needs to be pink the first few minutes of life, in fact there are strong indications this may be harmful. This question therefore was removed from the 2005/6 ILCOR guidelines [10]. In the 2010 ILCOR guidelines no firm recommendations for suctioning of babies with meconium stained amniotic fluid was given and consequently this fifth question was removed [1].

38.4 Guidelines for Resuscitation of the Newly Born

There are several sets of international guidelines for newborn resuscitation. The World Health Organisation (WHO) published their guidelines for basic newborn resuscitation in 1998 [11], which presently are under revision. These are applicable for most children around the world. The ILCOR guidelines are widely used internationally; these were first published in 1992 [12] and revised in 1999 [9], 2005 [10] and 2010 [1]. American Heart Association (AHA) and American Academy of Pediatrics (AAP) and European Resuscitation Council (ERC) in close collaboration with ILCOR produced their own guidelines [2, 3]. A number of countries have their own national guidelines modifying the ILCOR guidelines.

38.5 Physiological and Biochemical Changes

The physiological changes during asphyxia are well described in animal experiments.

Traditionally the asphyxial process has been divided into primary and secondary apnea. During primary apnea heart rate is still present even as high as 100 beats per minute (bpm) and there is cyanosis in mucus membranes and the skin. In secondary apnea the baby is pale and the heart rate is low or not present. In some guidelines different approaches are recommended for a primary and a secondary apneic situation. However, it is probably not wise to spend time to figure out in which of these stages the infant is. Preterm infants have a more rapid drop in heart rate than term infants and the distinction between primary and secondary apnea in the smallest infants probably is not very useful.

A metabolic and respiratory acidosis exists in newborns with birth asphyxia. Both animal and clinical studies have shown that base deficit is eliminated at a constant rate of 6-7 mmol/L/hour [13]. This elimination seems to be constant independently of the severity of birth asphyxia. Therefore, if the acid base status at birth is not known, this figure can be used to extrapolate back to the situation at birth. For instance if base deficit is 10 mmol/L at one hour of age it probably was approximately 16-17 mmol/L at birth. Such calculations can be useful for assessing prognosis and for court cases.

38.6 Initial Evaluation and Stabilisation

ABCD for resuscitation is the same whatever size of the patient.

- A: Airways should be cleared and the infant positioned correctly
- B: Breathing should be stimulated or performed
- C: Circulation should be assessed by heart rate and skin colour
- D: drugs, are rarely needed in the newborn.

38.6.1 Response to Birth

Resuscitation is a team work: optimally, two trained persons should actually perform the resuscitation and one should assist. Each team member should identify themselves and it should be clear who the team leader is. Each order should be stated clearly and repeated by the recipient.

As soon as the infant is delivered, a clock is started and, during the transport to the resuscitation table, the personnel should make a preliminary opinion regarding the seriousness of the situation. First and foremost the respiratory efforts are assessed and the heart rate is recorded. Heart rate should, after the short initial stabilization, be monitored more precisely. Bradycardia combined with no respiratory efforts or gasping are warnings of immediate intervention. Central cyanosis indicates insufficient oxygenation. Pallor may be a sign of reduced cardiac output, anemia, hypovolemia, hypothermia, or acidosis. Apgar scoring is not performed before 1 minute of life and in addition it takes some time. Apgar score therefore in itself should never be used as a criterion for resuscitation.

38.6.2 Initial Stabilisation

Start out by assessing the baby's response to birth and keep the baby warm and position it correctly; supine with the head close to the examiner, and keep airways clear.

Most infants require only to be delivered into a warm room where they can be dried immediately. The umbilical cord should be cut with sterile equipment and the breathing pattern should be observed. The mother herself is most often the best caregiver and provider of warmth, food and protection from infections.

Heat loss should be prevented and this is one important reason why deliveries should be performed in a warm room. For preterm infants a temperature in the room of 26°C is recommended. The newborn should be quickly dried with towels, giving particular attention to the drying of the head, and swept into preferably pre-warmed towels or other linens. It is recommended that the infant be placed supine under a radiant heater on a table designed for resuscitation with the head

toward the person performing resuscitation. Extremely low birth weight infants are put into plastic bags before dried. This secures a more stable temperature until the child is put into an incubator.

38.6.2.1 A - Airways

Stimulate the infant to breathe by drying. Airways are secured both by suctioning and by positioning the infant correctly in the supine position. There is however, no justification for the practice of routine suctioning in the mouth and pharynx or gastric emptying not even after C-section, because such suctioning might even be harmful. A child who starts to breath spontaneously within 10-15 seconds most probably does not need to be suctioned. In addition, it seems that the esophagus functions as a seal and a closed esophagus therefore helps blowing air into the lungs instead of the stomach during bag and mask ventilation. Opening the esophagus with a suctioning catheter will result in more air into the stomach in case of bag and mask ventilation. However, occasionally the mouth, nose, and pharynx should be suctioned to clear airways. Remember that the mouth should be suctioned before the nose to prevent aspiration if the newborn should gasp when his or her nose is suctioned. Suctioning deep in the pharynx or the larynx, especially the first 5 minutes of life, may elicit bradycardia and bronchospasm. Airways are best opened with the head in the "sniffing position", overextension should be avoided. Tactile stimulation as gentle rubbing of the back may be useful but do not shake the baby and do not waste time by tactile stimulating an apneic child.

38.6.2.2 B - Breathing

According to a recent study from United Kingdom in 2000 bag and mask ventilation was performed in 2.6% of all deliveries. This was a decrease from 3.9% in 1988 [6]. These figures are lower than given above from the USA and indicates that the need for bag and mask ventilation may vary throughout the world and is less than 5% in Western Europe. Still the number who would need bag and mask ventilation is high.

Clinical judgement for when to initiate ventilation should be based on heart rate, breathing efficiency, colour, and tone. However, the formal indications are very simple:

Start ventilation

- If the newborn is apneic or there is insufficient breathing movements and/or heart rate < 100 bpm
- If the newborn is breathing with heart rate > 100 bpm but is persistently cyanotic.

Apneic infants with heart rate > 100 bpm probably represent those with primary apnea, and they often require tactile stimulation and a few inflations with a bag and mask only. Apnea and heart rate <100 bpm may represent secondary apnea, and ventilation should be initiated. In most of these cases, the heart rate improves and spontaneous ventilation quickly develops. Ventilation, when needed, should start as soon as possible, preferably not later than 30 seconds after birth.

The recommended rate of ventilation according to the AHA/AAP is 40–60 breaths per minute. Inflation pressure of 30 cmH_2O or even greater may occasionally be needed for term babies in order to open the lungs. For preterm infants peak inspiratory pressure should be lower for instance 20 cmH_2O, however, depending on the size of the baby. The best way to monitor successful ventilation is to follow the heart rate which should be rapidly increasing. In addition, observe improvement in colour and muscle tone and movements. Recently it has been shown that it is difficult to make an assessment of colour [14], and in the most recent guidelines there is therefore less emphasise on colour assessment. Observation of the chest wall movements is difficult. Recent data indicate that tidal volumes may be too high when there is a clear rise and fall of the chest during the respiratory cycle. It has also been shown that assessment of tidal volume by observing chest rise is extremely difficult [15, 16].

Heart Rate

Traditionally a cut off for heart rate < or > 100 bpm has been used. This has been based on the assumption that a heart rate < 100 bpm in a newborn indicates bradycardia which in most cases is caused by asphyxia. It was demonstrated 2 decades ago that it takes 1 to 2 minutes to establish an efficient gas exchange in vaginally delivered babies and a few more minutes are needed after C- section [17, 18]. Because there is a relation between gas exchange and heart rate it may be normal not to achieve a heart rate of 100 bpm or more even at an older age than one minute. Supporting this are the recently published values for heart rate the first ten minutes of life in normal non-asphyxiated newborns with no medical intervention [19]. At one minute of age term infants have in mean a heart rate of 99 bpm with interquartile range of 66–132 bpm. For preterm infants the values are 96 (72–122) bpm. Recent studies indicate that the heart rate is underestimated by auscultation [20].

In term or near-term infants the pulse very quickly picks up during a successful resuscitation procedure for instance increasing from typically 90 beats per minute (bpm) at one minute of age to 110 bpm at 90 seconds of life. In babies with the lowest Apgar score heart rate is initially lower but typically increases with 20 bpm during the first 30 seconds of resuscitation [13, 21]. In infants < 30 weeks of gestation the heart rate increase is slower. It takes in median 73 seconds (inter quartile range 24–165 seconds) to reach a heart rate of 100 bpm and 243 (191–351) seconds to reach 120 bpm. The heart rate in such small babies is not stable until it reaches 120 bpm [22]. An adequate response to adequate ventilation therefore should for most term and near-term infants be ap-

proximately 20 bpm increase the first 30 seconds of bag and mask ventilation, however slower in preterm infants. After adequate ventilation has been established for 30 seconds, the heart rate should be recorded for not longer than 10 seconds. If the heart rate is at least 100 bpm and spontaneous respirations are established, positive pressure ventilation may be discontinued. If the heart rate is > 60 bpm and improving, ventilation should continue. If the heart rate is less than 60 bpm and not improving, assisted ventilation is continued and external heart massage is started.

Ventilation

A self-inflating bag, a flow-inflating bag, or a T piece resuscitator can be used. The use of a bag and mask requires experience and it is possible to practice on mannequins. By inserting a resuscitation monitor which is a hot-wire anemometer placed between a ventilation device and the face mask or endotracheal tube, the operator can minimize leaks by adjusting the face mask position to minimize leaks [23, 24]. In an Australian study an average of 60% leaks were found around the face mask, however after the operators adjusted the mask position the leak was reduced to 10% [24].

It is not easy to deliver an adequate tidal volume by bag and mask ventilation. However, it seems that starting ventilation in many instances stimulates the baby to breath because the inflation pressure induces the baby to make inspiratory efforts (head's paradoxical reflex) securing an adequate tidal exchange [25]. The optimal tidal volume for newborn resuscitation is not known. However, a range between 4–8 mL/kg is often recommended. Excessive tidal volume may lead to volutrauma [26], and a too low tidal volume leads to atelectasis and inadequate gas exchange. Both conditions may trigger cytokine release and inflammatory changes in the lung [27, 28].

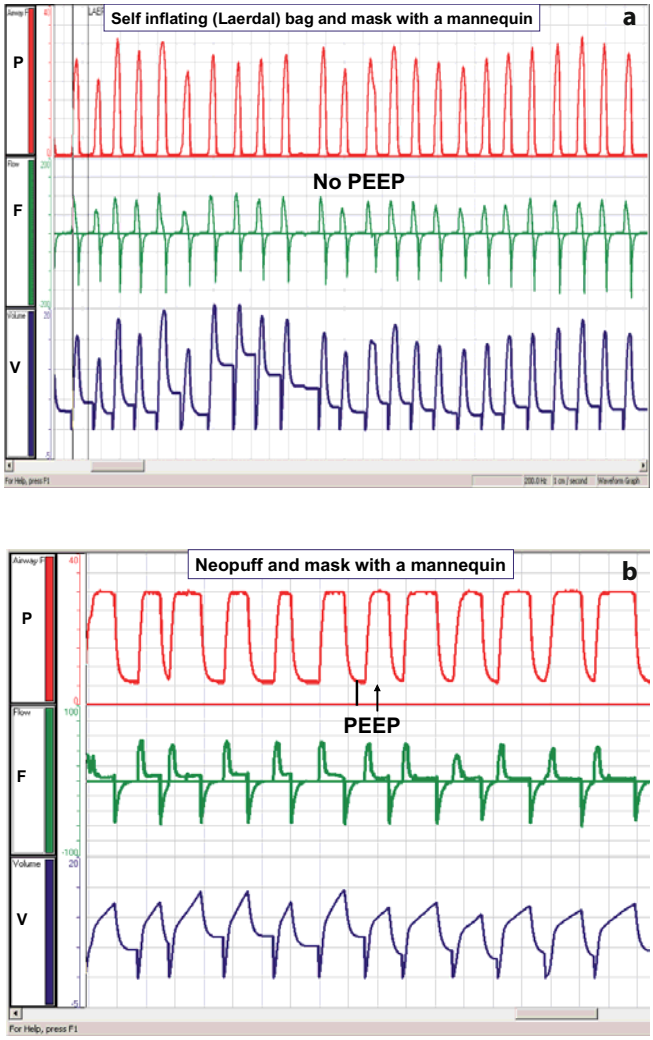

Fig. 38.1 **a** Ventilation of a mannequin with a self inflating bag. Notice the variable peak inspiratory pressure (*P*), flow (*F*), and volume (*V*), and the lack of positive end expiratory pressure (*PEEP*). **b** Ventilating a mannequin with a T-piece device. Notice the identical and exact peak inspiratory pressures (*P*), the presence of PEEP and the uniform flow and volume delivered

PEEP or CPAP?

In order to oxygenate the lungs must be ventilated to develop a functional residual capacity. Infants who do not breathe will need respiratory support. Positive end expiratory pressure (PEEP) has been tested out in animal models [28–30]. There is however, not much evidence regarding the use of PEEP or not in the delivery room. However, one randomised controlled trial in preterm infants showed that more infants were subsequently intubated and ventilated in a group without continuous positive airway pressure (CPAP)/PEEP compared with a group ventilated with a 10 second prolonged inflation followed immediately by CPAP/PEEP [31]. It is becoming more and more common to apply PEEP during ventilation in the delivery room. CPAP in many centres is applied immediately for low birth or extremely low birth weight infants (ELBWI). More and more centres are using a T-piece device when ventilating the newborn in the delivery room. This delivers a PEEP that can be adjusted to the wanted level. Fig. 38.1 shows recording when a mannequin is ventilated by the author with a self inflating bag (Fig. 38.1a) and a T-piece device (Fig. 38.1b). By using a T-piece device peak inspiratory pressure and volume are similar from breath to breath.

Studies have shown that a T-piece device delivers more exact peak inspiratory pressures than a self inflating or a flow inflating bag, in addition to delivering an exact PEEP.

In premature infants, especially in ELBWI, ventilation may be difficult because of airway obstruction. In such cases airways and the mask must be repositioned [23]. Fig. 38.2 illustrates leak during bag and mask ventilation of a 27 weeks gestation newborn [16].

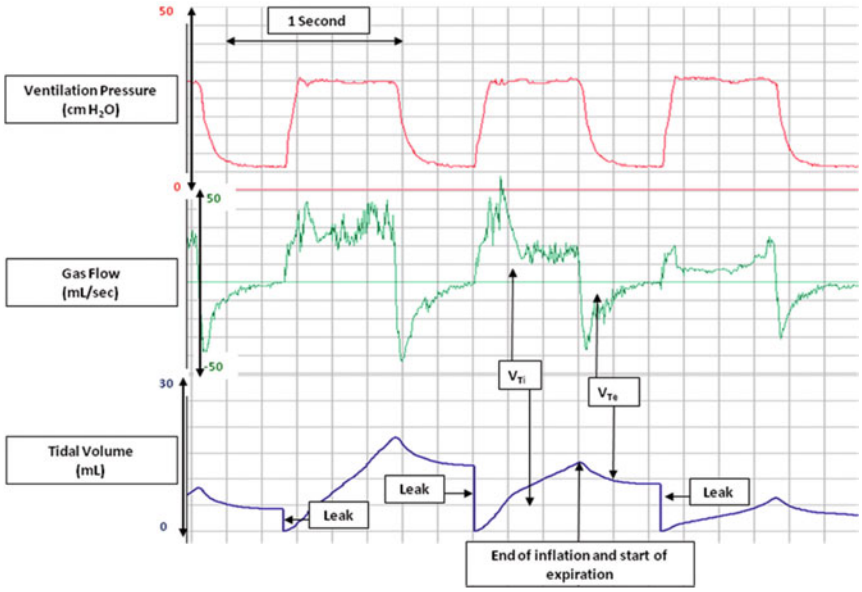

Fig. 38.2 Airway leak in a 27 week infant ventilated with face mask. The area underneath the inflation flow curve is greater than that under the expiratory flow curves. Leak is displayed as a straight line in the tidal volume curve. Notice the variable leaks. From [16], with permission

Endotracheal Intubation

Endotracheal intubation is necessary when bag-mask ventilation is not efficient to normalize the heart rate and establish spontaneous respiration. If chest compressions do not quickly improve heart rate or prolonged assisted ventilation is anticipated, endotracheal intubation should also be carried out. A newborn infant without any heart beat or respiratory movement should also promptly be intubated, as bag and mask ventilation probably is not very efficient. Some infants in secondary apnea also need intubation and the most immature infants who often do not have sufficient muscle strength for their respiratory drive. The ILCOR guidelines recommend that exhaled CO_2 is monitored for confirmation of tracheal tube placement by for instance a colorimetric detector. Their use and limitations have been described in the literature and is reliable in most cases (32–34). By using a resuscitation monitor a correct placement of the endotracheal tube can in most cases be established after the first breath by observing the gas flow in and out of the trachea [35, 36]. By observing

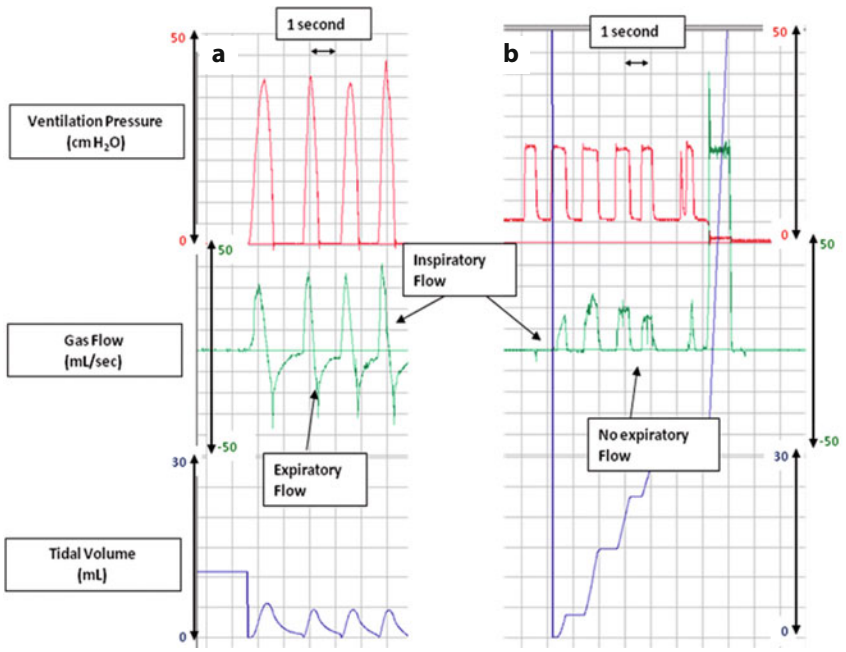

Fig. 38.3 **a** Correct endotracheal tube placement in a 26 week infant. Both inflation and expiratory flow curves return to baseline, indicating a correct placement of the endotracheal tube. **b** Esophageal tube placement in a piglet. The inspiratory flow curve returns to baseline indicating gas flow towards the endotracheal tube. In contrast there is no expiratory flow curve indicating endotracheal tube placement in esophagus. From [16], with permission

the leak it is also possible to make an assessment whether the size of the tube is correct or not. See Fig. 38.3.

Endotracheal intubation is rarely needed in term infants. In one study from USA it was carried out in approximately 1 per 1000 deliveries [5]. A study from UK has shown a decrease in the need of intubation between 1993-7 from 2.4% but still as high as 1.2%, of all live born [37]. This indicates a wide variation in intubation practise from approximately 1 per 100 to less than 1 per 1000 babies.

In another UK study the need of endotracheal intubation for all weight groups decreased in the period 1988 to 2000 from 0.51% to 0.07% [6]. However, for infants with birth weight < 1500 g this decrease was not very evident and their intubation need was 50 fold increased compared with babies with birth weight 3000–3500 g. If Apgar score at 5 minutes was 0–3 intubation rate was increased 234 folds. Even an Apgar score at 5 minutes of 4–5 increased the intubation rate 100 fold. Emergency C-section and breech delivery increased the need of intubation 2–3 fold [6]. This dramatic reduction in intubation rates indicates both a reduction in birth asphyxia and an increase in C-section, and also a more restrictive attitude regarding need of newborn intubation.

Oxygen Supplementation

High concentration of supplemental oxygen (80–100%) has usually been recommended by most textbooks and guidelines dealing with newborn resuscitation. There was, however, no scientific basis for such recommendations. New studies have shown that even the most depressed term or near-term newborn infants are adequately resuscitated with room air. Several meta-analyses [38, 39], now including ten studies enrolled more than 2000 newly born infants in need of resuscitation [39], have shown that early recovery is faster if resuscitation is carried out with ambient air instead of 100% O_2; time to first breath was registered 30 seconds earlier, heart rate at 90 seconds and 5 minutes Apgar score were higher [21]. More importantly is that neonatal mortality is significantly reduced with 30–40% in room air resuscitated babies [38, 39]. In a meta-analysis from European countries relative risk for neonatal death was in fact reduced 69% from 3.2% to 1.1% in those resuscitated with 21% compared with 100% oxygen [39]. For this reason 100% oxygen should be avoided as a routine for newborn resuscitation. The 2010 ILCOR guidelines state it is for term babies "best to start resuscitation with air". For babies < 32 weeks a blender is often needed and for babies with gestational age between 32–37 weeks the optimal initial FiO_2 is not known [1].

Recent animal data also show that the pulmonary resistance by and large is reduced as efficiently with 21% as with 100% oxygen [40]. In addition, it seems that the exposure of hyperoxia increases the reactivity of the pulmonary vessels subsequently increasing the risk of pulmonary hypertension [41].

As mentioned, above extremely low birth weight infants may need some oxygen to get started. However, some smaller studies have so far shown that 30% oxygen is at least as efficient as 90% oxygen. ELBWIs who need resuscitation therefore safely can be initiated with FiO_2 of 0.21–0.30 [42–44].

If room-air resuscitation is not successful within 90 seconds we still supplement with oxygen. In such case, it probably is optimal to monitor the arterial oxygen saturation by pulse oximetry. The arterial oxygen saturation at one minute of life in non-asphyxic term or near-term babies is typically 60–70% and may be as low as 40% the first 3–4 minutes of life. Within 5–7 minutes approximately 50% of these babies have reached a SaO_2 of 90%. After C-section the saturation is in general 2 minutes behind the values found in babies born after vaginal delivery [45]. In high altitude the SaO_2 is lower than at sea level, still it is recommended to start resuscitation with air also in such areas of the world.

The oxygen supply in all delivery units should have a blender, so that the oxygen concentration can be adjusted to the requirement. If justified to give supplemental oxygen it is recommended to start with not more than 30–40% oxygen and adjust according to the clinical response, preferably following oxygen saturation measured by pulse oximetry. It is in case recommended that oxygen saturation follow the 10th to 25th percentile of recently published nomograms for normal SpO_2 values the first minutes of life of non-asphyxiated newborn babies [46]. The aim is to reach a SpO_2 of 90% after 5–10 minutes in term and near-term infants and 85% in premature infants less than 32 weeks.

38.6.2.3 C - Circulation

Chest compressions are rarely needed and not included for basic newborn resuscitation. If bradycardia persists with a heart rate of less than 60 per minute and no signs of improvement after adequate ventilation, chest compressions should be started. This is needed in 0.5 to 1 per 1000 births [7].

Chest compression should always be carried out simultaneously with adequate ventilation [47]. Chest compressions and ventilatory rate of 3:1 should be synchronized giving 90 compressions and 30 breaths per minute. The two thumb technique is recommended [48]. An adequately performed resuscitation with chest compressions obviously requires at least two trained persons. Heart rate should be assessed after 30 seconds of well- coordinated chest compressions and ventilation. When the spontaneous pulse rate has reached 60 per minute, chest compressions should be discontinued and positive pressure ventilation is continued at a rate of 40–60 breaths per minute. There are not much data, if any, supporting why a ratio of 3:1 between compressions and ventilation is chosen for newborns.

If the cardiac arrest is not of asphyxial genesis, a ratio of 15:2 may be considered [1].

38.6.2.4 D - Drugs

When oxygenation is established through an adequate ventilation of the asphyxiated infant, it is extremely rare that drugs are needed. Epinephrine (Adrenaline) was in one study indicated in 1:1200 deliveries [8] and is indicated in asystole or in sustained bradycardia (Hr < 60 bpm) in spite of adequate ventilation and oxygenation and at least 30 seconds of coordinated chest compressions and ventilations. One study showed that even trained resuscitators are not able to give epinephrine earlier than 4–5 minutes of age [8]. Epinephrine (1:10,000 solution) is given as a bolus as rapid as possible preferably intravenously in a dose of 0.01–0.03 mg/kg (0.1–0.3 mL/kg) which may be repeated every 3–5 minutes if needed. If the endotracheal route is chosen [49] a higher dose can be used (0.05–0.01 mg/kg). Bradycardia due to insufficient ventilation technique should be corrected by adequate ventilation before administration of epinephrine. The optimal epinephrine dose for newborn resuscitation has not been studied systematically.

Volume Therapy

Hypovolemia is rare and volume infusion was in one study administered in 1:3000 deliveries, however hypovolemia was found in only 25% of these. Thus hypovolemia is an extremely rare event occurring in only 1:12,000 near-term or term infants [8]. Hypovolemic shock should be suspected if there is pallor; delayed capillary refill, weak pulses, persistently low heart rate, or the circulatory status does not improve in response to the treatment steps described above. Shock may be treated with repeated transfusions of volume expanders, usually 10 mL/kg. As volume expanders, normal saline or Ringer's lactate are recommended. The volume expander may be given over 5–10 minutes and repeated. Albumin or other plasma substitutes are not so much recommended any more. Blood volume expanders during acute resuscitation are only indicated when there are unmistakable signs of shock with evidence of acute blood loss, including feto-maternal hemorrhage. If there has been blood loss O-negative blood cross-matched with the mother's blood is given. Avoid rapid boluses of volume expanders or hyperosmolar solutions to premature newborn infants. In one experimental study in hypovolemic and asphyxiated newborn piglets the use of volume therapy seemed to be counterproductive [50]. Volume therapy therefore should be given on strict indications only.

Sodium bicarbonate, THAM or naloxone have no routine place in newborn resuscitation [1, 51].

38.7 Meconium Aspiration

If the amniotic fluid is meconium-stained, there is no evidence that suctioning the oropharynx before the thorax is delivered has any beneficial effects. There are today no indications that an infant with thick meconium-stained amniotic fluid benefits from routine intubation and suctioning.

The previous practise of tracheal suctioning does not seem to have reduced the incidence of meconium aspiration syndrome or mortality [52]. It is therefore not anymore recommended that suctioning of the nose, mouth and posterior pharynx be carried out thoroughly before delivery of the shoulder and thorax. According to previous guidelines the trachea should be suctioned in "non-vigorous" child. If the child is vigorous, the mouth and nose should be suctioned only and resuscitation proceeded as required. "Vigorous" is defined as a newborn having strong respiratory efforts, good muscle tone, and a heart rate greater than 100 bpm. In the 2010 ILCOR guidelines it is written that "the available evidence does not support or refute the routine endotracheal suctioning of depressed infants born through meconium-stained amniotic fluid" [1]. Thus, there has been a shift in recent years from a very active to a less active approach in these children.

38.8 Preterm Infants

There are no or very few studies on optimal resuscitation of premature infants. This means that even the extremely low gestational age newborn infants are often resuscitated according to the guidelines and principles established for term and near-term infants. Recent studies have shown that cardio-respiratory resuscitation of these in the delivery room is not futile and a more active approach seems therefore to be established [53] and this has lead to a more active approach [54–58]. It is also clear that preterm infants need intervention more often than near-term or term newborn infants. In one study from the USA 5% of babies with birth weight between 500 and 1500 g needed chest compressions and 4% received epinephrine [53].

Babies with gestational age < 28 weeks should be put into polyethylene wraps or a plastic bag up to their neck, before drying, in order to maintain the temperature [1]. They often need to be intubated given surfactant and in some centres they are immediately extubated, the so-called INSURE (INtubation SURfactant Extubation) approach [59]. Ventilation should be carried out using a PEEP of 5–6 cm H_2O and many authors recommend putting these babies directly on CPAP [60]. These infants should be handled as gently as possible. Avoid rapid boluses of fluid and quick changes in body positions.

Preliminary data indicate that the extremely low birth weight infant in need of resuscitation often would need a brief exposure of oxygen- for instance start out with 30% O_2 in order to get an adequate heart rate response [43–45]. More data are needed before firmer recommendation for these tiny infants are given.

38.9 Withholding and Withdrawing Resuscitation

In some cases, resuscitation should be withheld or withdrawn [61]. The present ILCOR guidelines state that resuscitation could be withheld for neonates at the margins of viability or those with conditions which predict a high risk of mortality or morbidity. Many centres do not resuscitate newborn infants with gestational age of less than 23 weeks. In earlier recommendations resuscitation was not recommended if there was anencephaly, bilateral renal agenesis, spinal muscular atrophy type I (Werdnig-Hoffmann disease) with neonatal onset, trisomy 13, or trisomy 18. However, according to the newest guidelines [1] and I agree in that, strict indications are not given for which newborns who should not be resuscitated. This author resuscitates and tries to stabilize infants with a gestational age of 23 weeks or more.

Often, neither the exact diagnosis nor the gestational age is known at birth. A liberal policy of resuscitation is recommended whenever doubt about the care of the individual infant exists. This allows the doctor to collect more information about the clinical status and prognosis of the child. This also allows one to inform the parents so that they can become prepared and participate in the discussion and decisions about subsequent therapy. In the new ILCOR guidelines [1] resuscitation of babies without any pulse and not giving response with heart rate increase within 10 minutes can be considered to be stopped because of very poor prognosis in these children. However in babies with Apgar score > 0 the prognosis is not so well known and resuscitation could continue longer for instance 20 minutes.

38.10 Post Resuscitation Care

After a successful resuscitation, heat loss should be prevented. The child should be labelled and frequently checked with regard to breathing efforts, respiratory rate, colour, and heart rate, signs of birth injury or malformations. If the resuscitation was brief and the situation was not too dramatic, the newborn could be monitored in the nursery provided an adequate respiration is established. The child could be placed so that skin-to-skin contact with the mother is obtained. However, this depends on the local conditions and the possibilities for adequate observation by a trained observer.

Even if the infant is not in need of artificial ventilation following resuscitation, the child is often brought to the intensive care unit for further close follow-up which includes monitoring of the heart rate, ventilation, determination of arterial pH and blood gases, treatment of any hypotension with volume expanders or pressors, appropriate fluid therapy, and treatment of any seizures. Any hypoglycaemia or electrolyte disturbances should be corrected. Breast-feeding should be encouraged, if possible, as soon as 1 h after birth. If the resuscitation was unsuccessful and the child died, the parents need a close follow-up.

Every baby who has been resuscitated, even briefly, is at risk and should be followed-up carefully both at short and long term. Blood glucose should be kept within normal ranges [62].

38.10.1 Hypothermia Therapy

The 2010 ILCOR guidelines recommend moderate hypothermia as a protective treatment to term infants with hypoxic ischemic encephalopathy. Hypothermia should in case be induced in a controlled way following written protocols [63]. In the delivery room it is important to avoid hyperthermia because this may augment any brain injury in severe asphyxia [64].

38.11 Documentation

It is useful if one attending person observes and immediately writes down the procedures performed. A thorough documentation in the medical record of all observations and actions and names of the participants is required before the resuscitation is complete. Each institution should keep records documenting the condition and procedures carried out at birth. Every institution that provides deliveries must develop its own standards for newborn resuscitation and a plan of action should exist. The personnel should gain and maintain skills in newborn resuscitation by training using manikins, and an evaluation of the training is necessary.

38.12 Conclusions

Newborn resuscitation is not yet fully evidence based. However, recent years a rapid development has occurred and a more evidence based practice has been established. Still there are numerous unanswered questions.

The optimal PEEP has not been determined. Today a low oxygen approach should be chosen for newborn resuscitation starting with air in near-term and term babies. However, there are still uncertainties regarding the use of oxygen supplementation for premature infants and which oxygen saturation one should aim at in term babies not responding adequately to the initial resuscitation. How to use supplemental oxygen during chest compressions is not known. The optimal adrenaline concentration and the effect of volume therapy are uncertain. Further, the optimal procedure for chest compressions is not defined. None of the guidelines discuss the importance

of pCO_2 and a moderate hypercapnia in order to restore cerebral blood flow. This should be an area of research for the coming years.

In spite of that there is general agreement that the most important is to start ventilation as soon as this is indicated. In most cases a few blows of air is sufficient to restore the heart rate and help the baby start breathing on its own. By avoiding non-evidence based detrimental procedures such as hyperoxygenation of the child the results will be much improved. "Give a breath – save a life" was the name of a large Indian campaign for newborn resuscitation launched in the 1990's. This is what it is all about. To give breaths and to save lives.

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39

Oxygen Toxicity

Giuseppe Buonocore, Rodolfo Bracci, Serafina Perrone and Maximo Vento

39.1 Introduction

Although the use of oxygen in the care of newborns dates since the eighteenth century [1] and the toxic effects of oxygen had been mentioned already at the end of the 19th century [2], the first evidence of a relationship between oxygen toxicity and neonatal diseases emerged in the early 1950s when retinopathy was observed in premature infants breathing high concentrations of oxygen [3]. At about the same time, the red cells of newborns were demonstrated to have increased susceptibility to oxygen damage [4]. Great advances in our understanding of toxic effects of oxygen were made in the years that followed, when oxygen toxicity was recognized to be due to the development of reactive oxygen species (ROS). The main ROS are the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), lipid peroxide (LOOH), peroxyl radicals $RO_2^{\bullet}$ and the hydroxyl radical ($OH^{\bullet}$). Other important radicals are the highly reactive electron delocalized phenoxyl radical (C_6H_5O) and nitric oxide (NO) [4]. The term ROS includes free radicals, which are atoms or molecules with one or more unpaired electrons. A free radical can be defined as any molecule capable of independent existence with one or more unpaired electrons. In addition, ROS encompasses molecules that can be defined as free radicals (e.g., anion superoxide) and others that are oxidizing species, relative to molecular O_2 but do not possess an unpaired electron (e.g., hydrogen peroxide) [5]. Free radicals may react with other radicals, the unpaired electrons forming a covalent bond. The resulting molecule may decompose other molecules into toxic products. Free radicals may react with non-radical molecules in free radical chain reactions, which are stopped by antioxidant molecules, enzymes or protein reactions. Superoxide anion [O_2^-] is the precursor of most ROS and a mediator in oxidative chain reactions. Dismutation of O_2^- by superoxide dismutase (SOD) produces H_2O_2 which in turn may be fully reduced to water by glutathione peroxidase (GSH-Px) and catalase (Cat) or partially reduced to hydroxyl radical [$OH^{\bullet}$]. The latter reaction is called the Fenton-Haber Weiss reaction and is catalyzed by reduced transition metals, particularly iron, but also copper and zinc [6]. There is no specific scavenger for this radical and, once released, $OH^{\bullet}$ reacts with lipoproteins, cell membranes, lipids, proteins, DNA, amino acids and other molecules causing structural and functional damage to theses structures. Since the $OH^{\bullet}$ is formed by the so called Fenton reaction which is dependent on non protein bound iron (NPBI), the conditions of intracellular or extracellular availability of NPBI is one of the most important source of ROS dependent tissue damage. Oxidative tissue damage may also be mediated by reactive nitroxide species [6]. The reaction product of NO and O_2^- is the unstable molecule peroxynitrite ($ONOO^-$) which is regarded as highly reactive [6].

Free radical reactions are a normal occurrence in living organisms and ROS are involved in a myriad of physiological reactions. The possible toxicity depends upon various conditions. For example, production of O_2^- by phagocytes is an important defense mechanism, but extracellular release of free radicals by activated phagocytes may be a mechanism of tissue damage during inflammation and, in turn, this event may be favorable or unfavorable depending on different conditions [7, 8]. Oxidative balance, therefore, is an intriguing question, and much recent research has increased our knowledge of the relations between ROS and human diseases.

39.2 The Oxidative Stress in Fetus and Newborn

An excess of ROS, in relation to detoxification capacity, is called oxidative stress, a term coined for the first time by H. Sies [9] and used to describe the imbalance between oxidants and antioxidants that is a potential cause of damage. If oxidative stress is mild, cell defenses may increase by a mechanism

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

which generally involves enhanced gene expression of ROS scavenging activities [10]. Severe oxidative stress is generally followed by cell injury which may proceed to necrosis or apoptosis [10].

Oxidative stress in the fetus and newborn may result from decreased antioxidants, increased ROS or both.

The main sources of ROS are NADPH oxidase reactions, hypoxia-reoxygenation (hypoxanthine-xanthine oxidase reaction: Fig. 39.1), hyperoxia and paradoxically hypoxia [6, 11]. Intracellular and extracellular presence of ROS is provided by activated phagocytes. ROS released inside phagocytes during infection and cytokine production is an essential defense mechanism. It also alters the extracellular oxidative balance and harms tissues since the cells undergo an efflux of O_2^- [12]. Opsonization and activation of phagocytes is also known to occur not only following infection but even as a consequence of asphyxia and particularly hypoxanthine-xanthine oxidase reaction and, therefore during hypoxia-reoxygenation [13, 14] (see Fig. 39.1).

Intracellular physiological generation of ROS can occur as a byproduct with mitochondria, peroxisomes, cytochrome P 450, and other cellular elements [11]. The discovery of the homologs of the cytochrome subunit of the NADPH oxidase, the NOX family, demonstrated the importance of ROS generation via NOX since these enzymes have been found in virtually every tissue [15] (see Fig. 39.2). The complex mechanism of ROS production has been extensively investigated in mitochondria where ROS generation contributes to mitochondrial damage in a range of pathologies [11]. An important factor involved in ROS production is the concentration of the enzyme or protein containing electron carriers that can exist in a redox form able to react with O_2 to form O_2^-. The concentration of these enzymes will vary with organism, tissue state, age and hormonal status [11]. It has been recently pointed out that many of non-mitochondrial enzymes are ubiquitously expressed and may contribute to ROS production in parallel with mitochondria. The enzyme activity of NOX family in which each of the NADPH homologs produces ROS may be responsible of oxidative stress in different cells under different stimulation [15]. In the CNS, hypoxia induced ROS generation has been demonstrated [16].

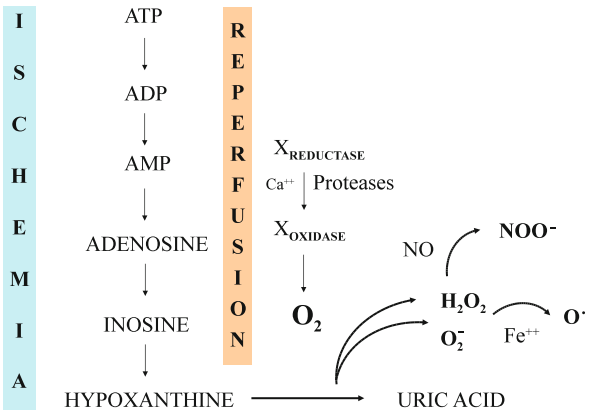

Fig. 39.1 Xanthine oxido-reductase system

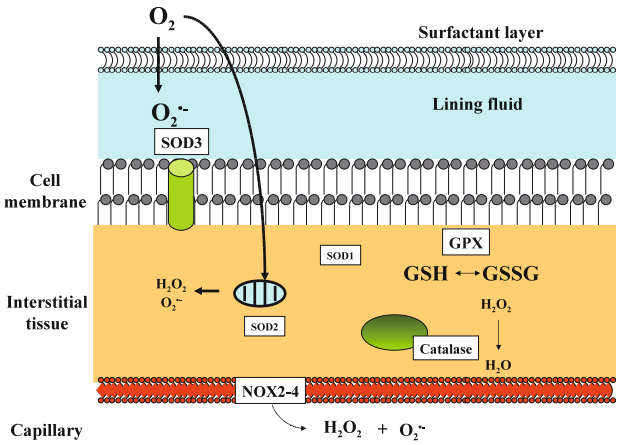

Fig. 39.2 Oxidative stress following hyperoxia

Oxidative stress following hyperoxia has been extensively investigated and has been recognized to be responsible for lung injury and possibly generalized tissue damage [17] (see Fig. 39.2). The sequence high inhaled oxygen (even for a short time), membrane bound NADPH oxidase activation, ROS production and DNA damage with cell apoptosis have been suggested as a consequence of exposure to high oxygen concentration [18]. Modification of neuronal nuclear membrane function leading to increased nuclear Ca^{++} influx, activation of Ca^{++}/Calmodulin dependent protein kinase, and protein mediated apoptotic protein expression has been reported in animals exposed to high oxygen concentration [19]. An important example of the various conditions capable of modifying ROS production and creating serious oxidative stress occurs in the endothelium. It is well known that endothelium dysfunction is mainly due to oxidative stress enhanced by various factors such as cytokines, ischemia re-oxygenation, glucose, LDL cholesterol, angiotensin II, shear stress, and vascular endothelial growth factor (VEGF) [20]. The occurrence of endothelium oxidative stress is important in neonatology, taking into account that endothelium is deeply involved in the infection mediated alteration of the coagulation system, and vascular troubles of neonate.

The risk of oxidative stress is enhanced in the neonate by the lower antioxidant capacity of the newborn and particularly the premature, in comparison with the adults. Free radical scavenger enzyme activities and many other components of the antioxidant system increase during intrauterine life and are low in the neonate [21, 22].

Of note is that the level and activity of the most-relevant antioxidant enzymes such as superoxide dismutases (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), and glutathione peroxidase (GPX; EC 1.11.1.9) change dynamically during development and mature in the last weeks of gestation, preparing the fetus for lung respiration [23–25].

In the fetal-to-neonatal transition, both blood oxygen content and oxygen availability abruptly increase in the first few minutes after birth to adult values, eliciting the generation of a burst of oxygen free radicals [26–28].

Free radicals in the fetal-to-neonatal transition may act as signaling molecules modulating maturation of specific metabolic pathways [29, 30].

However, in extremely premature infants, the combination of an immature antioxidant system plus a surfactant-deficient lung and the need for therapy with oxygen supplementation and mechanical ventilation together predispose to oxidative stress, lung-stretch damage, inflammation, and impairment of alveolar development, predisposing to chronic lung disease [31, 32].

However, generalized use of antenatal steroids has significantly reduced mortality and the incidence of severe complications in preterms younger than 34 weeks' gestation. Extremely low gestational age (ELGA) neonates with antenatal steroids spend less days on oxygen, on mechanical ventilation and have less severe complications such as bronchopulmonary dysplasia (BPD), patent ductus arteriosus (PDA), intra-periventricular hemorrhage (IPVH) or retinopathy of prematurity (ROP). Moreover, in spite of the increased antioxidant enzyme (AOE) activity and reduced oxidative stress and damage to proteins and DNA after antenatal steroid administration, the level of AOE activity was significantly lower than assessed in term infants or adults. Intriguingly, the response to antenatal steroids is conditioned by timing of administration before birth and by gender, with female neonates having a significantly increased response compared to male neonates [33].

Non-enzyme antioxidant factors are reported to be lower in the fetus and newborn than in adults and older babies [34]. One of the most studied antioxidant deficiency of fetus and newborn is tissue and erythrocyte low α-tocopherol. Other factors are important. Reduced glutathione (GSH) is the most abundant intracellular non-enzymatic antioxidant and is involved in the GSH-P and GSH-T enzyme activity. It has also complex interactions with environmental factors triggering oxidative stress [35]. Since in the cell the glutathione tripeptide is assembled from cysteine and glycine and then glutamate by consecutive actions of enzymes such as γ-cystathionase, γ-glutamylcysteine synthetase and glutathione synthetase, the possible problems with this mechanism may be due to recognizable inborn errors of metabolism or nutritional deficiencies [35, 36]. Several observations have shown that preterm infants have lower and age-related plasma concentrations of GSH and higher concentrations of glutathione disulfide (GSSG) than adults [37]. This occurrence may due to low GSH and low activity of GSH synthetase in the VLBW infants [36].

In this regard, there have been conflicting reports in the literature as to whether L-cysteine is an essential amino acid for the preterm infant as the result of the low activity of hepatic γ-cystathionase. Thus, studies performed in preterm infants revealed that plasma cystathionine concentrations in ELBW infants were significantly higher than in term infants, whereas cysteine concentrations were higher in term than in preterm. Furthermore, erythrocytes from preterm infants synthesize glutathione at a much lower rate than term infants when incubated with methionine, a process dependent on the γ-cystathionase pathway. These findings underscore the immaturity of the trans-sulphuration pathway and the conditional essentiality of L-cysteine in the parenteral and enteral nutrition of very preterm neonates. Moreover, it also explains the tendency toward oxidative stress [38].

Iron binding proteins have major antioxidant activity, protecting against metal induced $OH^{\bullet}$ production [39]. Therefore, among non-enzyme antioxidant factors the metal transporter proteins have an important function. In particular the transferrin concentration has a key role in protecting against the non protein bound iron (NPBI) promoted oxidative stress due to $OH^{\bullet}$ release [40, 41]. In this regard, low ceruloplasmin and transferrin may play an important role in neonatal susceptibility to oxidative stress and particularly to metal-induced $OH^{\bullet}$ production [42]. The finding of increased bleomycin-detectable iron in premature and some full-term infants is evidence of the real risk of increased pro-oxidant effects of iron in neonates [43]. High saturation of transferrin is therefore probably a risk factor for oxidative stress. It is generally accepted that NPBI is a pathological manifestation and it is extremely rare in adults [40]. Levels of NPBI in plasma of newborns are correlated with other markers of oxidative stress and are higher in hypoxic newborns [44]. Interestingly, plasma esterified F2-isoprostanes not only correlate with NPBI in the samples of cord blood of newborns but they increased after incubation of plasma with ammonium iron sulphate [45]. This observation strongly suggests the role of NPBI in enhancing oxidative stress in the neonate. High plasma NPBI has been found in hypoxic newborns with poor outcome [46]. Increased markers of oxidative stress such as hypoxanthine, total hydroperoxide and advanced oxidation protein products have been reported in hypoxic newborns and in preterm neonates at birth and on the seventh day of life [47, 48]. Increased F2 isoprostanes have been also found in growth restricted fetuses suggestive of oxidative stress and hypoxia [49]. These observations demonstrate that oxidative stress follows hypoxia with a mechanism of hypoxia re-oxygenation, NADP oxidase reactions or, paradoxically, by release of ROS by hypoxic cells.

Oxidative stress is involved in tissue damage induced by infection and sepsis. Although there is general agreement that neonatal phagocytes, especially those of premature infants, have abnormalities of various functions, active neonatal secretion of pro-inflammatory cytokines suggests a very complex situation in which oxidative stress following infection may be more dangerous in newborns, especially if premature, than in adults [50].

The key role of oxygen toxicity in the development of the retinopathy has been recognized from more than half a cen-

tury. The complex mechanism of oxygen toxicity is also discussed in Chapter 146.

Oxygen toxicity is particularly harmful for the lungs. The mechanism of damage is complex. Lung injury may be caused directly by ROS production in response to hyperoxia or indirectly by ROS due to phagocyte activation and inflammation. The two mechanisms seem to be integrated. The particular toxicity of ROS in the immature lung is mainly due to the low antioxidant capacity of premature infants, as well as to the possibly high toxicity of ROS in rapidly developing tissues [51]. Increased production of ROS under certain conditions may also play a role. The main sources of ROS production in the immature lung are ischemia, reperfusion, phagocytosis and hyperoxia [52–54].

The lung is highly susceptible to high oxygen concentrations especially when administered with positive pressure. In a prospective clinical study ELGA neonates were randomly assigned to become ventilated with higher (90%) and lower (30%) oxygen concentrations as initial inspiratory fraction of oxygen. Of note is that those neonates receiving more oxygen load during fetal to neonatal transition had a higher oxidative stress as detected by a decreased GSH/GSSG ratio. Moreover, urinary determination of ortho-tyrosine/phenylalanine ratio and 8-oxo-dihydroguanosine/2 de-oxyguanosine ratio revealed that premature infants who received more oxygen had increased oxidation of circulating proteins and higher damage to nuclear DNA. In addition, markers of inflammation (interleukin-8 [IL8] and tumor necrosis factor-α [TNF-α]) were evenly increased. Interestingly, infants with increased markers of oxidative stress and inflammation in the first days of life developed with significantly higher incidence of BPD and stayed more days on oxygen and mechanical ventilation [54].

Effects of hyperoxia in the lung and a close relationship between oxidative stress and inflammation have been demonstrated [55, 56]. Magnetic resonance studies have confirmed the previously reported experimental data on the consequences of hyperoxia in the lungs of premature newborn animals, namely edema, congestion, immune cell infiltration and decreased number of alveoli per square meter [57]. The finding of a large number of neutrophils and high concentrations of IL-8 and leukotrienes in bronchopulmonary lavage of infants with severe chronic lung disease (CLD) demonstrates the role of inflammatory reactions and ROS production in the development of this disease [58].

Oxidative stress is involved in the increased hemolysis and anemia of the newborn and particularly of the premature. The red cells of newborns appear to be more susceptible to the toxic effects of oxidative stress than those of adults [22] and the free iron content of red cells, a risk factor for hemolysis, is higher in newborns in comparison with the adults [59]. It is interesting that during aerobic and also hypoxic incubation, increased O_2^- production, free iron release and senescence antigen production showed the highest significant differences between adults and neonates [60]. This finding suggests that hyperoxia and hypoxia may generate red cell damage via ROS and possibly have a role in the increased hemolysis in the neonate.

In conclusion, experimental studies and clinical observations demonstrated high susceptibility of the fetus and newborn to oxidative stress. Increased release and decreased detoxification in the newborn appear to be negatively correlated with the gestational age.

39.3 Clinical Aspects and Prevention

39.3.1 Diagnostic Procedures

ROS have an important role in the development of retinopathy, chronic lung disease, necrotizing enterocolitis, renal failure, hemolytic anemia, septic shock and also brain damage. The clinics of these pathologies are treated in the corresponding chapters.

Several methods have been proposed in the attempt to detect the presence and the severity of the oxidative stress. Some frequently used approaches such as the measurement of erythrocyte antioxidant defenses are not accurate since the red cell enzyme activities are age dependent and values may be an expression of an old or young red cell population [61].

39.3.2 Lipids

The thiobarbituric acid (TBA) test should not be used to measure lipid peroxidation because most of TBA-reactive material is not related to lipid peroxidation. Measurements of malondialdehyde (MDA) by HPLC, 4-hydroxynoneal and particularly isoprostanes are more accurate [61]. The most work has been done with F-2 isoprostanes which arises from arachidonic acid non-cyclo-oxygenase (COX) peroxidation. Neuroprostane or F-4 isoprostanes has also been measured [61].

39.3.3 Proteins

Detection of oxidation of proteins can be obtained by measurement of carbonyl groups and the use of proteomics appears to a promising method in order to identify specifically oxidized proteins [61]. Assay of total hydroperoxides represents a measure of overall oxidative stress given that they are the intermediate oxidative products of lipids, peptides and amino acids [61]. Allantoin and 8-hydroxydeoxyguanosine can be measured in plasma and urine and the levels are high in conditions of oxidative stress [62, 63]. Nitrosyn levels have been reported as a marker of $ONOO^-$ production from leukocyte

activation [64]. A very specific, useful and non-invasive mean to monitor oxidative damage to circulating proteins and amino acids is the measurement of ortho-tyrosine. Oxidation of phenylalanine produces three different metabolites, ortho, para and meta-tyrosine, which can be detected in urine by high performance liquid chromatography coupled to mass spectrometry [65]. Interestingly, only ortho-tyrosine is produced by non-physiologic metabolic pathways and derives from hydroxyl derived oxidation of phenylalanine. In recent work, a significant correlation between the amount of oxygen given during resuscitation and the urinary elimination of ortho-tyrosine been detected; moreover, the increased antioxidant capacity of preterm human milk has also been assessed by this methodology [66, 67].

39.3.4 Intracellular Redox Status

A major marker of oxidative stress is the GSH/GSSG ratio which directly reflects alteration of intracellular redox status since when the oxidative stress exceeds the reductive capacity of GSH reductase the cell loses glutathione exporting the excess of oxidized glutathione [35]. NPBI levels appear to be a very reliable marker of oxidative stress, particularly in the newborns as previously indicated. Measurements of ethane in expired air may be useful in detecting oxidative stress [61].

39.3.5 DNA Damage

Determination of oxidized bases of DNA have been carried out by HPLC coupled to MS/MS [67].

39.3.6 Prevention of ROS Tissue Damage

Avoidance of conditions such as infections, asphyxia, hyperoxia and retinal light exposure, under which excessive free radical release occurs, are the best defense against development of imbalances in pro-oxidant and antioxidant factors in the neonate. Frequent reports of NPBI in plasma of neonates suggest that indiscriminate iron supplementation should also be avoided.

The concept of optimal oxygenation of newborns has recently been revised in order to clarify whether the optimal oxygen saturation unanimously accepted for normal infants and adults is also the best for sick neonates, especially if premature.

In an attempt to avoid the risk of tissue damage caused by ROS in the first hours of life, when susceptibility to oxidative stress is particularly high, the effects of O_2 were investigated. As previously reported, hyperoxia-induced lung injury has been demonstrated. Indications on the use of oxygen were recently reported by Cow et al [68] in a 5-year study in a tertiary neonatal center where oxygen therapy was adjusted to optimize neonatal care and decrease the incidence of ROP. They recommended avoidance of repeated increase and decrease in FiO_2 in response to the oxygen saturation monitor and maintenance of oxygen saturation within "acceptable" limits. They also recommended setting an alarm for oxygen saturation below 85% and above 93% for newborns under 32 weeks of age. More recently, comparison of two populations of high risk newborns kept at O_2 saturations of 88–98% and 70–90% showed a significant reduction in ROP in the group at lower O_2 saturation but no differences in mortality or poor outcome [69]. Data from clinical outcome seem to demonstrate that low birth weight infants cared for with liberal approaches had more cognitive disability compared with those cared for with more restrictive approaches after 10 years [69]. Data collected from the Oxford Vermont Network in extremely low birth newborns demonstrate significant lower incidence of chronic lung disease and ¾ ROP among babies carried with target oxygen saturation of <95% compared to those kept at more than 95% [70]. Shulze et al [71] did not observe signs of mismatch between systemic oxygen delivery and demand in low birth weight infants kept at 93–96% O_2 and 89–92% saturation. However, Poets et al [72] reported higher incidence of ROP in infants < 30 weeks' gestation using oxygen saturation limits of 80–92% in comparison with 92–97%. Therefore, the relationship between Hb oxygen saturation, risk of oxidative stress and oxygen toxicity is still a problem. Even a recent Cochrane review failed to define the target range for maintaining blood oxygen levels in preterm/LBW infants [73]. A subsequently repeated Cochrane review declares that "the question of what is the is the optimal target range for maintaining blood oxygen levels in preterm LBW infants was not answered by the data available for inclusion in this review" [74].This is presumably due to the complex mechanism of oxygen toxicity which may be expressed at different percentages of O_2 saturation under different conditions.

Conventional indications suggest that optimal oxygen tension should be maintained between 50 and 70 mmHg [75]. However, it should be taken into account that the ability of pulse oximetry to reliably detect hyperoxia remains controversial and it has been shown that oxygen saturation more than 90% can be associated with an arterial oxygen tension of more than 80 mmHg [69].

Hyperoxia and oxidative stress may occur during neonatal resuscitation (see Chapter 38).

39.3.7 Protective Effect of Human Milk in the Preterm Infant

The antioxidant properties of human milk are reported widely in the literature.

In a recent study Friel et al [76] confirmed that human milk of term and of preterm mothers provided better antioxidant protection. They found that human milk produced less ascorbate whether or not oxidative stress was present. Elevated concentrations of ascorbate are prooxidant in the extracellular milieu. Moreover, the antioxidant enzyme activity of catalase, superoxide dismutase, and glutathione peroxidase increased with time in human milk. Additional studies have shown that human milk-fed neonates had a higher plasma total antioxidant and vitamin C content. Biomarkers of oxidative status, such as total peroxide and oxidative stress index, were higher in formula-fed neonates at 3 to 6 months' postnatal age [77]. Human milk of preterm mothers at 35–36 weeks' post-conceptional age also seemed to have a positive effect on the oxidant status of their infants. In a recent study, preterm infants fed preterm human milk have significantly lower elimination of markers of hydroxyl radical aggression to protein and DNA, meaning that they produce lower amounts of free radicals.

Thus, in the clinical setting even in the acute stage preterm infants should be fed early with human expressed milk, which offers an additional protection against free radicals [78].

39.3.8 Antioxidants

Substances inhibiting phagocyte activation or xanthine oxidase and arachidonic acid metabolism, or decompartmentalizing free iron and making it available for the Fenton reaction, have also been investigated, together with those scavenging ROS directly or repairing ROS-induced membrane injury, like calcium antagonists and beta-blockers. The subject has been reviewed by Buonocore and Groenendal [79].

On the whole, the results obtained in newborns have been uncertain. Several antioxidant substances have been used in newborn animals and humans in an attempt to improve the worst prognosis of damage, presumed to be due to ROS. Many, such as superoxide dismutase (SOD), showed the same disadvantages in newborns. Other drugs, such as allopurinol and desferrioxamine have shown good results in animals, but no advantages in human newborns.

Among the antioxidants, melatonin has a special place since it has been reported to have several interesting effects such as enhancement of antioxidant enzyme activities and neutralization of H_2O_2 singlet oxygen and peroxynitrite; it appears to scavenge $OH^{\bullet}$ [80]. Administration of melatonin has been reported to decrease the concentrations of pro inflammatory cytokines and markers of oxidative stress in RDS and in septic newborns [81, 82]. In the animals, a study of Carloni et al [83] demonstrated that melatonin has a long-lasting beneficial effects on brain damage following hypoxia ischemia occurring during the development. However, the effects of the melatonin may be due to a combined actions since the melatonin in addition to the antioxidant effects, possesses also endocrine, autocrine and paracrine activity and appears to decrease the inflammation.

Vitamin E is a powerful, widely tested antioxidant. Conclusions of a recent Cochrane review are that vitamin E supplementation to preterm infants reduces the risk of intracranial hemorrhage but increases the risk of sepsis; in very low birth weight infants it seems to reduce the risk of retinopathy and blindness [84]. There is no evidence to support the use of vitamin E at high doses or the aim of keeping serum tocopherol levels above 3.5 mg/dL [85]. It should be remembered that some formulations of vitamin E are poorly adsorbed [86].

Peroxidation products in stored lipid emulsions have been shown to increase lipid peroxidation *in vivo* in newborns and adults. Since parenteral nutrition has been associated with increased formation of ROS, it is necessary to protect intralipids from light and add vitamins [87]. Finally, although hyper-bilirubinemia can be regarded as a natural antioxidant factor, the risk of brain damage due to high plasma levels of this molecule, particularly in premature babies, should not be underestimated.

39.4 Conclusions

The mechanism by which the oxidative stress is deeply involved in the development of several diseases of the fetus and newborn is complex and probably not yet fully understood. However, multiple observations strongly suggest that increased free radical and ROS play a considerable role as well as the deficiency of antioxidant agents. In particular, hyperoxia and inflammation as well as the episode of hypoxia reoxygenation appear to be a source of increased ROS release which may cause tissue injury, particularly in the preterm infant, either by direct effect or as a consequences of endothelium dysfunction.

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40

Physical Examination of the Newborn

Claudio Fabris and Alessandra Coscia

40.1 Introduction

A complete physical examination of every newborn should be performed by a trained neonatology care provider within 24 hours of birth. Although there is no international standard, routine examination is regarded as a good practice in the guidelines for postnatal care [1–4].

Aims of the routine neonatal examination are:

1. to detect problems arising from maternal or familial diseases or from complications of labor and delivery;
2. to check for conditions suspected during antenatal period (e.g., congenital malformations) and to provide information about management;
3. to detect any acute condition requiring urgent diagnosis and therapy;
4. to diagnose congenital problems not already identified at birth (e.g., congenital heart disease, developmental dysplasia of the hip);
5. to screen some specific conditions, such as developmental dysplasia of the hip and congenital cataract;
6. to provide initial health and educational advice for the parents (e.g., breastfeeding, SIDS prevention, hemolytic disease of the newborn (HDN) prevention, safe transport in cars, vaccination).

The first quick examination should be performed immediately after birth, in the delivery room. This first step, mainly based on inspection, should check that the infant looks well and there are no pathological conditions requiring parents to be informed (e.g., congenital malformations or any doubt about the baby's gender) or immediate management (e.g., respiratory distress). A careful examination of the newborn should be routinely performed in presence of the mother or both parents, in a quiet warm room, with adequate lighting.

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40.2 Full Examination

Before introducing himself to the mother, the health care provider must check basic information regarding maternal (e.g., maternal age, socioeconomic status, smoking, drug or alcohol abuse, any chronic disease) and family history and previous obstetric history. It is very important to inquire if there were any complications in the pregnancy and to know the results of pregnancy screening tests and diagnostic procedures (e.g., ultrasound examinations, amniocentesis). It is necessary to get information about problems during labor and delivery, as well as about the baby's gender, birthweight and gestational age. All these items should be documented in the infant's medical record.

The newborn can be properly examined clothed only in the nappy. There is not any strict rule regarding the order of the examination, but some tips can be given. It would be better first to perform a general "gestalt", whole inspection and then continue with a systematic review (e.g., from head to foot), as well take advantage of any period of quiet or sleeping to check the anterior fontanelle, to examine the eyes, to perform the auscultation of the heart and the lungs and the

Table 40.1 Sequence for an appropriate full evaluation of the newborn

1.	General observation, including posture, tone and movements
2.	Measurement of head circumference, length and recording them, together with the weight
3.	Skin (color and characteristics)
4.	Head (facial appearance, fontanelle)
5.	Examination and auscultation of heart and lungs
6.	Femoral pulses
7.	Palpation of abdomen
8.	Eyes, ears, nose and mouth
9.	Neck, including the clavicles
10.	Arms, hands, legs, feet
11.	Genitalia and anus
12.	Evaluation of nervous system
13.	Hips
14.	Prone position: back and spine

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

palpation of the abdomen. It is better to leave the examination of the hips until the end, because the Ortolani-Barlow test is unpleasant for the newborn. Regardless the order of examination, the health care provider must make sure he has not omitted any item and he must record all findings. A suggested sequence is given in Table 40.1.

Many conditions observed during the examination are very common and they have little clinical importance or usually resolve spontaneously. On the other hand, there are some conditions, less frequent, that require further investigation or urgent therapy.

40.2.1 General Observation and Measurements

General observation includes weight, length and head circumference measurement: percentiles have to be ascertained from weight charts. If there is any doubt regarding gestational age, it should be determined by a scoring scheme [5, 6]. Simple observation allows short arms and legs to be noticed; these can indicate the presence of skeletal dysplasia.

Healthy term babies spend most time in quiet or active sleep, but transition between behavioral states can be rapid: crying is an usual finding during the examination [7]. A healthy full-term infant usually lies in flexion position (flexion of the arms at the elbows and of the legs at the knees), with a relatively symmetric limb position and posturing: however, the presentation at birth can influence posture in the first days of life. Asymmetric tonic neck reflex can be noted if the head is turned to one side: in this case, there is extension of the ipsilateral arm and leg and flexion of the controlateral extremities. Infants manifest spontaneous motor activity, which consists of moving their limbs in an alternating fashion [8].

40.2.2 Skin

The skin of a healthy newborn should be reddish pink, but peripheral cyanosis (hands, feet and circumoral area) is a common finding during the first 48 hours, as well traumatic cyanosis, often associated with petechiae, affecting the presenting part. Some conditions may be present:

- *Central cyanosis*: this is best observed on the tongue and it requires urgent investigation for pulmonary disease or congenital heart malformations. Plethoric newborns can appear cyanotic because they have more than 5 g per 100 mL of reduced hemoglobin.
- *Pallor*: when generalised it can be associated with anemia or shock.
- *Jaundice*: appearing within the first 24 hours of life, jaundice most often indicates hemolysis and requires further investigation and treatment.
- *Capillary hemangioma* (stork bites): these pink macules appear on the upper eyelids, the mid forehead and the nape of the neck: those on the forehead and eyelids disappear or fade over the first year, whereas those on the neck are covered with hair.
- *Neonatal urticaria* (erythema toxicum neonatorum): white papules appear on erythematous skin, usually on the second or third day of life and migrate to different sites.
- *Milia*: retention of keratin and sebum may cause these benign white cysts distributed on the nose and cheeks.
- *Mongolian blue spots*: they are blue-black maculae, more frequent in African-American or Asian babies, located at the base of the spine or on the buttocks, and in some cases also on the legs or in other parts. They fade slowly over the first few years of life.
- *Harlequin color change*: this is a vasomotor instability which causes a longitudinal reddening down one half of the body and a blanching down the other side.
- *Edema*: this may be generalized or localized: if present, it is always pathologic in the newborn. Pedal edema may be a sign of Turner syndrome.
- *Port-wine stain* (nevus flammeus): a vascular malformation of the capillaries in the dermis and usually evident at birth, whereas strawberry nevi (cavernous hemangioma) appear only after 1–2 months of life. When port-wine stain affects distribution of the trigeminal nerve, it may suggest underlying intracranial vascular malformations (Sturge-Weber syndrome), whereas large lesions on the limbs may be associated with bone hypertrophy (Klippel-Trénaunay syndrome).

40.2.3 Head and Face

Head dimensions and shape should be evaluated and fontanelle and sutures should be palpated. The head can be considerably moulded during labour and delivery. Babies in the breech presentation in utero often have the so-called "breech head" (prominent occipital shelf).

There may be some transient phenomena:

– bruising and abrasions from the use of forceps, vacuum extractor or scalp electrodes;
– caput succedaneum, edema and bruising of the presented part, that extends beyond the sutures;
– cephalhematoma, a collection of blood between the periosteum and the skull bone that does not extend beyond the sutures and usually affects parietal bone.

The anterior fontanelle can measure up to 3 × 3 cm, but its dimension can be variable: its tension (when the baby is not crying) may indicate raised intracranial pressure, from cerebral edema, intracranial hemorrhage, hydrocephalus or meningitis. After delivery the coronal sutures are often overriding, but ridging at the suture lines implies premature fusion of the sutures (craniosynostosis).

Asymmetry is a frequent finding due to pressure in utero and during labor and delivery. Palpation of the skull bones can reveal areas of craniotabes, caused by pressure in utero from maternal pelvis, that occur in 2% of normal full-term newborns and more frequently in the preterm baby. Cutis aplasia (small defect of the scalp) is a congenital malformation with risk of bleeding or infection of the dural venous sinuses and it may be a clue to the presence of a syndrome. Most newborns resemble one or other parent, but when the face appears unusual, the examiner should search for other dysmorphic manifestations, to exclude a syndrome.

40.2.4 Ears

General shape, size and position of the ears have to be evaluated. Malformations can be associated with hearing loss and require a hearing check. Preauricular pits, skin tags anterior to the ear and accessory auricles may be associated with an increased risk of renal abnormalities [9, 10]. Low-set ears (when the top of the pinna falls below a line drawn from the outer canthus of the eye at right angles to the face) are a characteristic of several syndromes.

40.2.5 Nose

Newborns have to breath through their nose, so complete nasal obstruction (i.e., from choanal atresia) causes intense respiratory distress and requires prompt investigation and treatment. If there is any doubt, a fine catheter should be passed through each nostril to ensure that both nares are patent. Flaring of the alae nasi is always abnormal and indicates the presence of respiratory distress that requires further investigation.

40.2.6 Eyes

The eyes should be examined both by inspection and with an ophthalmoscope. If the lens is opaque from a congenital cataract or glaucoma, the red reflex cannot be elicited: any abnormality of the red reflex requires urgent evaluation by an ophthalmologist. Congenital cataract is the commonest form of preventable childhood blindness. Swelling of the eyelids is common, a mucoid discharge ("sticky eye") is often present in the first 2 days of life and usually resolves spontaneously. A purulent discharge, accompanied by redness and swelling of the eyelids, requires microbiological investigation and treatment. Subconjunctival hemorrhages are very common due to delivery and usually resolve within 1–2 weeks. The iris is normally blue or grey in the newborn. Partial defect is called "colobomata" and it could be associated with a defect in the retina, complete absence is called "aniridia" and it is associated with an increased risk of Wilms' tumor. Size of the cornea has to be checked: if the corneal diameter is greater than 13 mm, there might be a congenital glaucoma.

40.2.7 Mouth

Direct inspection of the inside of the mouth is important, in order to exclude cleft palate, even mild defects (submucous cleft or posterior cleft palate). Other harmless findings may be seen, including Epstein pearls (small white pearls of microkeratosis along the midline of the palate), mucus-retention cysts of the gums (epulis) or on the floor of the mouth (ranula), short frenulum ("tongue tie"). These conditions usually do not need treatment. Natal teeth (rare in Caucasian race) are best removed.

40.2.8 Neck

The newborn has a relatively short neck, but a very short neck may indicate abnormalities of the cervical spine (Klippel-Feil syndrome). A webbed neck is one of the characteristics of Turner syndrome, and posterior redundant skin may suggest Down syndrome. Palpation of sternomastoid muscle may be evidence of "tumors", caused by bleeding or ischemia, sometimes resulting in fibrosis. Clavicle fractures may occur during delivery with shoulder dystocia: it is possible to palpate a lump on the clavicle that results from a callus around the fracture and usually recovers without treatment.

40.2.9 Examination of Chest and Auscultation of Heart and Lungs

A certain degree of breast enlargement is normal in newborns of either sex and a few drops of milk ("witch's milk") may be discharged.

Neonatal routine examination detects only a half of the infants with congenital heart disease, even if the baby has significant structural heart lesion. At birth, the pressure difference between left and right side may be still low and then newborns with ventricular septal defects might not have a heart murmur. Moreover, ductus-dependent lesions can present symptoms only a few days after birth, and femoral pulses are often palpable at first examination [11, 12].

Cardiorespiratory state can be deducted by simple inspection (respiratory rate and signs of respiratory distress, such as retractions or grunting). If cyanosis is present, pulse oximetry should be performed: pulse oximetry is currently not recommended as routine practice [13–15]. Normal respiratory rate

is 40–60 breaths/min: if a baby has a rate persistently above 55 breaths/min, he needs to be carefully evaluated. In a full-term newborn, there should be no grunting, no retractions and no flaring of the alae nasi: if present, they are symptoms of respiratory distress.

Palpation of the precordium may detect thrills or a pronounced ventricular heave. Peripheral pulses, in particular femoral pulses, should be always palpate: patent ductus arteriosus produces a bounding pulse, whereas absence or difficulty to feel femoral pulses suggests coarctation. If coarctation is suspected, it can be confirmed by comparing blood pressure in the arms and legs: a difference of 20 mmHg or more suggests coarctation. A heart murmur may be heard, but most are innocent: 60% of normal newborns have a systolic murmur at 2 hours of life: the origin of an innocent murmur may be the acute angle at the pulmonary artery bifurcation, patent ductus arteriosus, or tricuspid regurgitation [16]. Features that suggest a significant murmur are as follows [17]: pansystolic; ≥ 3/6; harsh quality; best heard in the upper left sternal border; abnormal second heart sound; and difficult to feel femoral pulses.

Electrocardiogram or chest X-ray rarely change the clinical diagnosis [18]. Due to the availability of ultrasound evaluation, this evaluation, with expert consultation, should be performed in any infant with a suspected significant murmur.

40.2.10 Abdomen

Simple observation may reveal any anomaly. The umbilicus is a common source of infection, so any discharge or reddening of the skin requires attention. A single umbilical artery occurs in 0.3% of newborns and is associated with an increased risk of congenital malformations, in particular renal abnormalities. Ultrasound of the renal tract should probably only be reserved for babies who have other problems, other than a single umbilical artery [19]. An umbilical hernia is a common finding, especially in African-American babies.

A diastasis of the recti is frequently appreciated by palpation, the liver edge is usually palpable 1–2 cm below the right costal margin. Also the spleen tip and the kidney are palpable: it is important to detect abnormal renal mass or enlargement of the bladder, which may be a sign of urinary outflow obstruction in a male infant.

40.2.11 Genitalia

The position and the tone of the anus must be checked and passage of meconium should be recorded.

Male Hydroceles occurs very frequently in the neonatal period, and usually resolves spontaneously. Inguinal herniae are common, in particular in preterm babies. The penis should be checked for length (usually about 3 cm) and position of urethral meatus. Sometimes the penis looks short, but a normal organ is buried in sovrapubic fat. An abnormal position of urethral meatus indicates hypospadia (glandular, coronal, mid-shaft or perineal): ventral curvature reveals the presence of a chordee. Glandular hypospadia without chordee does not require any treatment. In a full-term baby, testes should be palpable, but about 6% of newborns have one testis undescended at birth: if at 3 months of age the testis is still undescended, a pediatric surgeon or urologist should be consulted. Neonatal testicular torsion usually occurs before birth: neonatologists should consult a pediatric surgeon, although the testis has usually already undergone infarction [20].

Female At full term the labia majora cover the labia minora, in preterm babies the clitoris and the labia minora appear prominent. There is often a white or bloodstained discharge, due to maternal hormone withdrawal. Small hymenal skin tags, mucoid cysts or a ring of vaginal mucosa are common.

40.2.12 Spine

Swelling, dimples, a hairy patch or a nevus along the spine or over the base of the skull might indicate underlying vertebral, spinal cord or brain anomalies. Simple sacrococcygeal pits are common and harmless, simple dimples (less than 2.5 cm from the anus, less than 5 mm wide, without other cutaneous signs) do not indicate occult dysraphism [21–23].

40.2.13 Limbs

Extremities are examined to inspect shape, posture, symmetry and size. About 45% of individuals with Down syndrome have single transverse palmar creases, but the same finding may occur in 4% of the Caucasian and in 16.8% of the Chinese population. Polydactyly is often a familial trait, but it may be caused by a dysmorphic syndrome. Digital remnants should be removed surgically.

Observation of spontaneous arm movements and testing passive movements may indicate a fracture or a brachial plexus lesion. Lack of active movement and pain on passive movements indicates a fracture or an infection. In a brachial plexus lesion there is initially a lack of movement in the arm. After 48 hours, in an upper root palsy (Erb's palsy: C5, C6 and sometimes C7), the arm is internally rotated, without active abduction. In a complete palsy of upper and lower roots (Klumpke's palsy: C5, C6, C7, C8 and T1) the arm flails and there may be ptosis and Horner's syndrome, the hand may be clawed [24]. Most brachial plexus lesions

recover spontaneously: if recovery doesn't occur by 3 months of age, the baby should be referred to a specialist [25, 26].

40.2.14 Hips

The incidence of developmental dysplasia of the hip (DDH) and instable hip is about 1–2 per 1000 births and 10 per 1000 births respectively. The risk of DDH is increased in some conditions: female sex, breech presentation, positive family history, oligohydramnios, any abnormality of the lower limb, any other anomaly suggesting intrauterine compression (i.e., torticollis) [27]. Clinical screening of DDH (Ortolani's and Barlow's tests) has not reduced the number of babies requiring surgery (0.7 per 100 live births) [28]: moreover, most infants with abnormal neonatal examination have normal hips. Clinical examination by an unexperienced examiner is only little better than no screen [29].

There is another screening strategy, ultrasound screening, which is best performed by a skilled operator before 3 months of life: this can also detect clinically stable but anatomically abnormal hips. No universal agreement exists concerning the best DDH screening method. Many clinicians suggest that clinical neonatal examination is the best strategy, if performed by properly trained examiners and followed by selective ultrasound, based on risk factors [30]. In the Ortolani-Barlow manoeuvres, the baby lies supine, with the legs relaxed. For Ortolani's test, first the examiner should straighten out the legs and then flex the hips to a right angle: after placing the middle finger over the greater trochanter of each leg and the thumbs over the internal aspects of the thighs, the examiner slowly abducts and externally rotates the hips: a definite "clunk" indicates that a previous dislocated femoral head has slipped into the acetabulum [31]. The next manoeuvre is Barlow's test, which allows dislocation of an unstable hip. Holding the hips and knees as in Ortolani's test, with the hips at about 70° abduction, the examiner presses forward and medially each hip in turn: if the hip is dislocated, the femoral head slips into the acetabulum with a "clunk" [32].

40.3 Neurological Examination

Formal testing is only required in newborns with neuromuscular problems. The evaluation should be performed with minimal discomfort to the infant and the main goal is to exclude any suspicious neurological signs [8].

Neonatal neurological evaluation includes the following tests (see also Chapter 130):

- general observation
- level of consciousness and behavioral states
- mechanical signs: head, spine, limbs
- motor system: muscle strength and tone, spontaneous movements
- reflexes (deep tendon reflexes and primitive reflexes i.e., Moro, grasp, suck, root)
- autonomic function (heart and respiratory rate, bladder and bowel function).

Motor system function can be assessed by testing skeletal muscle posture, tone and movements.

Full-term infants usually exhibit flexion of the limbs. Muscular tone consists of an active or passive resistance to stretch and it results from a complex interaction between agonistic and antagonistic muscles: then any alteration in muscle tone could represent a sign of a variety of underlying neurological diseases.

The assessment of muscle tone includes the observation of posture and movement, the evaluation of resistance to passive movements and the production of active or passive movement by specific maneuvers, such as pull-to-sit manoeuvre and ventral suspension. In the pull-to-sit manoeuvre, as the infant is pulled up from the supine position by his wrist, there should be some flexion of elbows and slight flexion of neck, with the head coming up almost in line with the body. In the ventral suspension, as the newborn is hold in the air with the examiner's hand under the chest, flexed arms and the head lie almost on a plane with the body for a few seconds.

Increased resistance to passive movement may indicate hypertonicity, often associated with an opisthotonic posture and obligate extension during ventral suspension.

Babies with reduced resistance to passive movements and unrestricted movements usually tend to drape over the examiner's hands in the horizontal plane: these findings indicate hypotonia, whereas weakness is a reduction in muscle power or strength, and it may occur together or not with hypotonia. Hypotonia combined with weakness usually indicates an involvement of peripheral nervous system, whereas hypotonia without weakness indicates an involvement of the central nervous system (brain or spinal cord).

Rooting, sucking, grasp and Moro responses are the most important primitive or developmental reflexes: they should disappear in the first months of life. When they persist, an underlying central nervous system dysfunction should be suspected.

Crossed extension, placing and stepping reaction are other primitive reflexes.

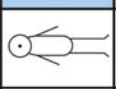
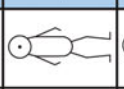
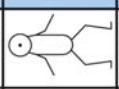
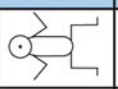
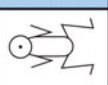
The main aim of the neurological evaluation in the routine examination is to exclude the presence of suspicious neurological signs. The findings that require further evaluation are the following:

- persistent hypotonia, paucity of spontaneous movements
- abnormal posture (frog posture, opisthotonus, excessive fisting, fisted thumbs)
- asymmetric movements
- failure to suck
- high-pitched cry
- persistent and extreme irritability
- convulsions
- lesions over the spine.

MATURATIONAL ASSESSMENT OF GESTATIONAL AGE (New Ballard Score)

NAME _____ SEX _____
HOSPITAL NO. _____ BIRTH WEIGHT _____
RACE _____ LENGTH _____
DATE/TIME OF BIRTH _____ HEAD CIRC. _____
DATE/TIME OF EXAM _____ EXAMINER _____
AGE WHEN EXAMINED _____
APGAR SCORE: 1 MINUTE _____ 5 MINUTES _____ 10 MINUTES _____

NEUROMUSCULAR MATURITY

NEUROMUSCULAR MATURITY SIGN	SCORE -1	0	1	2	3	4	5	RECORD SCORE HERE
POSTURE								
SQUARE WINDOW (Wrist)	 >90°	 90°	 60°	 45°	 30°	 0°		
ARM RECOIL		 180°	 140–180°	 110–140°	 90–110°	 <90°		
POPLITEAL ANGLE	 180°	 160°	 140°	 120°	 100°	 90°	 <90°	
SCARF SIGN								
HEEL TO EAR								
							TOTAL NEUROMUSCULAR MATURITY SCORE	

SCORE
Neuromuscular _____
Physical _____
Total _____

MATURITY RATING

SCORE	WEEKS
-10	20
-5	22
0	24
5	26
10	28
15	30
20	32
25	34
30	36
35	38
40	40
45	42
50	44

GESTATIONAL AGE (weeks)
By dates _____
By ultrasound _____
By exam _____

PHYSICAL MATURITY

PHYSICAL MATURITY SIGN	SCORE -1	0	1	2	3	4	5	RECORD SCORE HERE
SKIN	sticky friable transparent	gelatinous red translucent	smooth pink visible veins	superficial peeling & / or rash, few veins	cracking pale areas rare veins	parchment deep cracking no vessels	leathery cracked wrinkled	
LANUGO	none	sparse	abundant	thinning	bald areas	mostly bald		
PLANTAR SURFACE	heel-toe 40–50 mm: -1 < 40 mm: -2	>50 mm no crease	faint red marks	anterior transverse crease only	creases ant. 2/3	creases over entire sole		
BREAST	imperceptible	barely perceptible	flat areola no bud	stippled areola 1–2 mm bud	raised areola 3–4 mm bud	full areola 5–10 mm bud		
EYE / EAR	lids fused loosely: -1 tightly: -2	lids open pinna flat stays folded	sl. curved pinna; soft; slow recoil	well-curved pinna; soft but ready recoil	formed & firm instant recoil	thick cartilage ear stiff		
GENITALS (Male)	scrotum flat, smooth	scrotum empty faint rugae	testes in upper canal rare rugae	testes descending few rugae	testes down good rugae	testes pendulous deep rugae		
GENITALS (Female)	clitoris prominent & labia flat	prominent clitoris & small labia minora	prominent clitoris & enlarging minora	majora & minora equally prominent	majora large minora small	majora cover clitoris & minora		
							TOTAL PHYSICAL MATURITY SCORE	

Fig. 40.1 Assessment of gestational age by revised Ballard method. Reproduced from [6], with permission

40.4 Assessment of Gestational Age

The most reliable guide for assigning gestational age is an early antenatal ultrasound, combined with the mother's last menstrual period: early antenatal ultrasound has 95% confidence intervals of less than 7 days. Several methods can be used to assess gestational age: those based on physical criteria, those based on neurological development and those that combine physical and neurological examination.

Physical criteria progress in an orderly fashion with increasing gestational age and can be assessed immediately after delivery. Neurological criteria include the assessment of posture, passive and active tone, reflexes and righting reaction, and require the infant to be in an alert rested state, not so easy to obtain until the second day of life.

The most accurate method to assess gestational age is to combine the physical criteria and the neurological assessment: Dubowitz and Dubowitz scoring system involves 11 physical criteria and 10 neurological findings [5], whereas simplified Ballard system includes six physical and six neurological criteria to shorten the time taken (Fig. 40.1). The new Ballard score is valid for extremely premature infants [6, 33].

In any case, these methods are accurate only to ± 2 weeks, with an overestimation in extremely premature infants.

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41

Primary Investigations in the Term and Preterm Newborn

Ignazio Barberi, Eloisa Gitto and Diego Gazzolo

41.1 The Term Newborn

41.1.1 Newborn Screening

Newborn screening, started in 1960, is primarily directed at disorders in which clinical complications develop during the immediate postnatal period.

41.1.1.1 Metabolic Screening

Metabolic diseases result from biochemical abnormalities that are manifest during the first days after birth [1]. Phenylketonuria (PKU) was the first metabolic disorder known to benefit from early diagnosis and immediate dietary treatment. Infant with PKU were identified early, in large numbers, and developed normal while receiving treatment [2]. The success of such screening led to further progress in the investigation of additional tests for metabolic diseases, such as galactosemia, maple syrup disease (MSUD), and homocystinuria. These tests could be applied to the same blood specimen obtained for PKU screening. In 1990, tandem mass spectrometry (MS/MS) started a new era in newborn screening [3]. The technology allows for the detection of more than 30 biochemical genetic disorders with a single assay with high specificity and with a very low of false positive rate. Currently, the disorders detected by most MS/MS newborn screening programs can be divided into three major categories: amino-acid disorders (including urea cycle defects), organic acid disorders and fatty acid oxidation defects [3].
In 1999, the American Academy of Pediatrics (AAP) Newborn Screening Task Force recommended that Human Resources and Services Administration (HRSA) developed and implemented nationally recognized newborn screening system standards and policies in collaboration with the Maternal and Child Health Bureau (MCHB) of HRSA and the American College of Medical Genetics (ACMG) [4]. Criteria included: 1) the current availability of an effective and rapid screening test; 2) an efficacious treatment and adequate understanding of the natural history .

Metabolic screening varies from country to country and each nation applies its own program of neonatal screening. A multidisciplinary report by the ACMG, HRSA and AAP recommended a panel of 29 tests and there is universal newborn screening for metabolic disorders in, many developed countries [5] (Table 41.1).

Conditions most commonly screened for are congenital hypothyroidism, galactosemia, adrenal hyperplasia (cah), biotinidase deficiency, sickle cell disease, cystic fibrosis are performed by immunoassays, radioimmunoassay (RIA), fluoroimmunoassay (FIA), and enzyme-linked immunosorbent assay (ELISA) techniques. The topic is considered in more detail in Chapter 120.

41.1.2 Infections

A wide variety of infectious agents may affect newborn such as bacteria, viruses, fungi, protozoa, and mycoplasmas. This topic is considered in more detail in Chapters 112–118.

Several laboratory tests have been evaluated for their ability to predict which at risk infants may develop symptomatic or culture–proven sepsis, but there is at present no single test that is sufficiently reliable. Investigators have proposed different combinations of laboratory tests but these various combinations were not more diagnostic than single laboratory tests although more predictive for negative cases [6].

Newborns at risk of sepsis need careful physical examination and standard laboratory investigation may be useful such as: WBC differential count, immature-to-total neutrophil (I:T ratio) and C reactive protein (CRP) [6]. Although

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Table 41.1 Newborn screening panel recommended by ACMG expert panel [4]

MS/MS detectable disorders
Organic acids
– Isovaleric acidemia
– Glutaric aciduria 1
– 3-Hydroxy-3-methylglutaric aciduria
– Multiple carboxylase deficiency
– Methylmalonic acidemia due to mutase deficiency
– 3-Methylcrotonyl CoA carboxylase deficiency
– Methylmalonic acidemia due to cobalamin A and B defects
– Propionic acidemia
3-Ketothiolase
Fatty acid oxidation
– Medium chain acyl-CoA dehydrogenase
– Very long chain acyl-CoA dehydrogenase
– Long chain 3-hydroxyacyl-CoA dehydrogenase
– Trifunctional protein deficiency
– Carnitine uptake defect
Amino acids/urea cycle
– Phenylketonuria
– Maple syrup urine disease
– Homocystinuria
– Citrullinemia
– Argininosuccinic aciduria
– Tyrosinemia type 1
Disorders detected by other methods
Hemoglobinoathies
– Hb SS-Sickle cell anemia
– Hb S/Beta-thalassemia
– Hb S/C disease
Others
– Congenital hypothyroidism
– Biotinidase deficiency
– Congenital adrenal hyperplasia
– Galactosemia due to GALT deficiency
– Congenital hearing loss
– Cystic fibrosis

sensitivity/specificity is limited, total WBC count and differential and I:T ratio can indicate bacterial infection. Taken together: 1) an elevated WBC is not predictive of infection in newborn infants; neutropenia is more common than neutrophilia in severe neonatal sepsis, but also occurs in association with maternal diseases (PIH, IUGR); 2) thrombocytopenia is a nonspecific indicator of infection; 3) longitudinal WBC monitoring in the first 24 hours from birth is more predictive of infection than a single determination [7–9].

CRP is an acute-phase reactant that increases in the presence of inflammation caused by infection or tissue injury. Raised levels of CRP are found in bacterial sepsis or meningitis. After inflammation onset, CRP synthesis increases within 4–6 h, doubling every 8 h, and peaks at about 36–50 h. Levels remain elevated with ongoing inflammation, but with resolution they decline rapidly due to a short half-life of 4–7 h. CRP demonstrates high sensitivity and negative predictive value. A single normal value of CRP at birth cannot rule out infection because the sampling may have preceded the rise in CRP. Serial CRP measurements may be helpful [10–12].

For asymptomatic term infants with positive laboratory tests, blood culture and antibiotic therapy are recommended until cultures are negative. Asymptomatic infants born before 35 weeks with risk factors for sepsis should have blood cultures before antibiotic therapy is started and before the results of laboratory tests. The duration of therapy should be guided by the baby's clinical condition and the results of the tests [13].

41.2 The Preterm Newborn

Preterm birth affects more than 500,000 babies in the United States each year [14]. In Italy the incidence of preterm birth is 6.7 % at <36 weeks of gestation, 5.2% between 32 and 36 weeks of gestation and 0.9% <32 weeks [15]. Preterm birth is one of the leading causes of infant mortality and morbidity [16]. It accounts for more than 70% of neonatal deaths and almost half the children with long-term neurological disabilities were born prematurely [17]. Since 1981, the US preterm birth rate has increased from approximately 9% to 12% [14, 18]. Research indicates that preterm birth is a multifactorial disease caused by genetic, social and environmental factors, which most likely interact to increase risk [19–26]. There are striking disparities in the rates and consequences of preterm birth across racial and ethnic groups in the US that are unexplained [18].

41.2.1 The Problem of Preterm Labor

The factor(s) controlling the spontaneous onset of labor are not known. This is frustrating from a physiological point of view, and also a major clinical problem. The last trimester of pregnancy is necessary for the maturation of the fetal lungs and other organs in preparation for extrauterine life. If this process is interrupted by an early delivery the chances of survival of the newborn are severely decreased. The mortality rate according to different countries still remains higher and constant, at lower gestational ages, with a wide range morbidity and disability in the surviving infants [27, 28]. The factors responsible for parturition in women remain unknown and the endocrine paradigms described above do not fit primates. Therefore, more research is necessary to identify the physiological trigger for parturition and to understand why the process may be activated prematurely in some pregnancies. It is also necessary to investigate the biochemical mechanisms regulating uterine smooth muscle activity in order to develop more effective and selective therapy to control uterine contractions when this is indicated. Critical clinical findings are reported in Table 41.2.

Table 41.2 Critical assessment findings for hypo- and hyperthermia

Hypothermia
- Pale, mottled skin that is cool to touch babies
- Acrocyanosis
- Respiratory distress
- Apnea, bradycardia, central cyanosis
- Irritability initially
- Lethargy developing as hypothermia worsens
- Hypotonia
- Weak, cry and suck
- Gastric residuals, abdominal distension, emesis
- Shivering in more mature
- Metabolic acidosis
- Hypoglycemia
- *And temperature measurement*!!

Hyperthermia
- Reddened skin that is warm to touch
- Tachypnea
- Tachycardia
- Irritability, lethargy, hypotonia, weak cry
- Poor feeding
- Apnea
- Sweating in more mature babies
- Dehydration

41.2.2 Neonatal Assessment at Birth

Based on potential multiorgan damage due to prematurity, close monitoring, in the early phases after birth, is essential. Primary investigations include vital signs recordings such as temperature determination, heart and respiratory rates, oxygen saturation monitoring in parallel with blood pH assessment.

41.2.2.1 Temperature Determination

Temperature determination is always required and might be recorded at 30 minutes intervals up to thermostability achievement. Furthermore, it is suggested that temperature be recorded at 1–3 hour intervals by continuous monitoring with axillary determinations every 1–2 hours [29]. In this regard, recordings should include environmental temperature (i.e., air temperature in the incubator or radiant warmer settings). Measuring the skin and core temperature simultaneously may be of help differentiating between fever as a result of disease and environmental overheating. Noting that the baby's servo-controlled skin temperature is relatively stable but that the environmental temperature has dropped also may be indicative of fever as the incubator responds to the high probe reading by cooling the infant's environment.

41.2.2.2 Cardiorespiratory Monitoring

The electrical activity of an infant's heart is picked up by chest leads (usually three), placed on the infant and recorded by a cardiorespiratory monitor. The recording is displayed on a visual screen as the infant's electrocardiographic pattern. The infant's respiratory pattern also is recorded, because the chest leads electronically detect movement of the infant's chest with each respiration. The oxygen saturation monitor relies on adequate perfusion to the site and the ability to detect arterial pulsation; thus if it is placed distal to a blood pressure cuff, there will be an inaccurate reading while the cuff is inflated. Newer models of pulse oximetry reduce the artefact that results from motion and low perfusion [30, 31]. These newer models also are indifferent to ambient light whereas older models were affected by light sources such as phototherapy.

41.2.2.3 Blood Pressure Monitoring

Monitoring of blood pressure is essential for the optimal management of premature infants. Blood pressure is one of the most important physiological parameters to evaluate clinical stability in critical care. Several medical conditions in premature infants determine changes in blood pressure (i.e., infections, drugs, dehydration, blood loss etc.). The recognition and treatment of abnormal blood pressure states has significant prognostic implications in neonatal intensive care [32]. Blood pressure in the newborn is related to weight and gestational age. Blood pressure rises with postnatal age, 1–2 mmHg/day during the first week and 1 mmHg/week during next 6 weeks in the preterm infant, just as in the full-term infant. The measurement of blood pressure can be obtained by non-invasive or invasive methods. In stable infants or when only intermittent blood pressure measurements are required, non-invasive methods are preferred. Blood pressure invasive monitoring is obtained via catheter that has been introduced into an artery. This method is indicated in very small or instable infants, especially those with severe hypotension. For more details, see Chapter 78.

41.2.2.4 Transcutaneous Blood Gas Monitoring

PCO_2 and PO_2 are important monitoring parameters in neonatal intensive care units (NICU). Compared to conventional blood gas measurements that cause significant blood loss in preterms, transcutaneous (tc) measurements allow continuous, non-invasive monitoring of blood gas levels [33]. Preterm infants are vulnerable to alterations in arterial oxygen or carbon dioxide tension . Changes in oxygen supply contribute to the subsequent development of retinopathy of prematurity or bronchopulmonary dysplasia. Hypocarbia has been associated with the subsequent development of periventricular leucomalacia and cerebral palsy, and may cause retardation of retinal vascularization [34]. Transcutaneous measurement of oxygen ($PtcO_2$) and carbon dioxide ($PtcCO_2$) tension is a non-invasive method that has recently offered some promise. Several studies have shown a good correlation between tc and arterial values [35, 36].

41.3 The Preterm Baby

41.3.1 Specific Risks for the Preterm Neonate

Preterm infants usually show multiorgan failure in reverse proportion to the gestational age.

The main target-organs are:

- central nervous system (CNS): including apnea of prematurity, intracranial hemorrhage, development disability, and cerebral palsy;
- retinopathy of prematurity (ROP);
- cardiovascular complications may arise from the failure of the ductus arteriosus to close after birth: Patent ductus arteriosus (PDA) is typical in preterm babies. Other congenital cardiac malformations will further analyzed in Chapter 76;
- respiratory problems that can range from lung malformations (pulmonary hypoplasia, see Chapter 66) to wet lung and finally to chronic lung disease (see Chapter 65);
- gastrointestinal and metabolic issues can arise from glycemia and ion disorders, feeding difficulties, and finally necrotizing enterocolitis (NEC). However of particular interest are kidney diseases that are extensively described in Chapters 124 and 125;
- hematologic complications including anemia of prematurity, thrombocytopenia, and hyperbilirubinemia, and (jaundice) that can lead to Kernicterus (for review see Chapter 83);
- neonatal sepsis including viral and bacterial forms that can be characterized by general (septicemia) or localized sepsis (pneumonia, urinary tract infection, meningitis etc.).

41.3.2 Patent Ductus Arteriosus (PDA)

The ductus arteriosus' function in the unborn baby is to allow blood to bypass the lungs, because oxygen for the blood comes from the mother and not from breathing air. In full-term babies, the ductus arteriosus closes shortly after birth, but it frequently stays open in premature babies. When this happens, excess blood flows into the lungs and can cause breathing difficulties and sometimes heart failure. More details about PDA and other congenital cardiac disorders are analyzed in Chapter 80.

41.3.3 Respiratory Distress Syndrome

Clinical, diagnostic and therapeutic strategies in RDS management are described in Chapter 62. One of the most common and immediate problems facing premature infants is difficulty breathing. Although there are many causes of breathing difficulties in premature infants, the most common is called respiratory distress syndrome (RDS). Its incidence is inversely proportional to gestational age and occurs most frequently in infants of less than 1200 g and 30 weeks' gestation. RDS affects male infants twice as frequently as in female infants (2:1) In RDS, the infant's immature lungs fail to produce surfactant and its administration is highly required according to RDS severity. Prophylactic and or rescue surfactant administration modalities are reported in Chapter 70.

In RDS respiratory drive recording is generally irregular in rate and depth, and is chiefly abdominal, rather than thoracic, with a rate of 30–60 breath/min. Tachypnea, a rate above 60 breaths/min after the first hour of life, is the earliest symptom of respiratory diseases. Periodic respirations are cyclic respirations of apnea (5–10 seconds) and ventilation (10–15 seconds) [37]. Apnea is a non-breathing episode lasting longer than 20 seconds and accompanied by physiologic alterations. Use of accessory muscle of respirations is indicative of a marked increase in the work of breathing. Retractions reflect the inward pull of the thin chest wall on inspiration. Retracting is best observed in relation to the sternum (substernal and suprasternal) and the intercostals, supracostal, and subcostal spaces. The increased negative intra-thoracic pressure necessary to ventilate the stiff, non-compliant lung causes the chest wall to retract. This further compromises the lung's expansion. The degree of retraction is directly proportional to the severity of the disease. Nasal flaring is a compensatory mechanism that attempts to take in more oxygen by increasing the size of the nostrils and thus decreasing the resistance (by as much as 40) of the narrow airways. Grunting is forced expiration through a partially closed glottis.

Because the clinical presentation of many respiratory and non-respiratory diseases is the same, a chest X-ray examination may be the only way to differentiate cause and establish the proper diagnosis. Measurement of arterial blood gases is used to demonstrate alterations in oxygenation and acid-base balance and to differentiate between respiratory and metabolic components.

41.3.4 Hyperbilirubinemia

A common treatable condition of premature babies is hyperbilirubinemia, which affects 80% of premature infants. Although mild jaundice is fairly common in full-term babies (about 60%), it is much more common in premature babies. In premature infants, the bilirubin peak usually is on the fourth–fifth day of life and may be 10–12 mg/dL, possibly rising to greater than 15 mg/dL without any specific abnormality of bilirubin metabolism. Therefore for bilirubin screening it is enough to take sample blood on the fourth–fifth day of life. Neonatal jaundice is completely analyzed in Chapters 81 and 82.

Table 41.3 Indications for routine monitoring of blood glucose for prevention of neonatal hypoglycemia

Maternal conditions
– Presence of diabetes or abnormal glucose tolerance test
– Preeclampsia and pregnancy-induced or essential hypertension
– Previous macrosomic infants
– Substance abuse
– Treatment with beta-agonist tocolytics
– Treatment with oral hypoglycemic agents
Neonatal conditions
– Prematurity
– Intrauterine growth restriction
– Perinatal hypoxia-ischemia
– Sepsis
– Hypothermia
– Polycythemia-hyperviscosity
– Erythroblastosis fetalis
– Iatrogenic administration of insulin
– Congenital cardiac malformations
– Persistent hyperinsulinemia
– Endocrine disorders
– Inborn errors of metabolism

41.3.5 Hypoglycemia and Hyperglycemia

Recognition that preterm newborn are at risk for disturbances in glucose homeostasis is the most important step in preventing both hypoglycemia and hyperglycemia (Table 41.3). Maintenance of a neutral thermal environment is especially critical to minimize energy expenditure in those infants at risk for hyperglycemia. Other conditions associated with hypoglycemia, such as asphyxia and hypothermia, may be avoided through appropriate obstetric and neonatal intervention. When hypoglycemia is suspected, the plasma or blood glucose concentration must be determined immediately. Ideally this determination should be made with one of the laboratory enzymatic methods, such as the glucose oxidase or hexokinase method, but even bedside reagent test strip glucose analyzers (i.e., glucometers) can be used if the test is performed carefully with awareness of the more limited accuracy of these devices. A number of current references use 40–45 mg/dL as the lower limit of "normal" plasma glucose concentrations in the first 72 hours of life [38].

41.3.6 Intracranial Hemorrhage

Intracranial hemorrhage (ICH) can affect newborns of all gestational ages and often is clinically "silent". Among ICH, germinal matrix hemorrhage and intraventricular hemorrhage (GM-IVH) are the most common in the premature population (see Chapter 138). Routine screening for GM-IVH is performed in infants < 30 weeks or < 1250g at birth. The timing of hemorrhage occurrence is mostly within the first three days of life. "Late" hemorrhage (i.e., after 3 days of age) may be associated with pneumothorax and its restriction of venous return to the heart.

Indications for cerebral ultrasound scan are discussed in Chapter 132.

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42

Physiology of the Gastrointestinal Tract

Arieh Riskin, Carlo Agostoni and Raanan Shamir

42.1 Development of the Gastrointestinal Tract: Organogenesis and Function

The gastrointestinal tract develops from the primitive digestive tube that originates from the dorsal part of the yolk sac. Initially the yolk sac is attached to the midgut of the digestive tube, but as early as the fourth week of gestation the gut becomes distinct from the yolk sac. The yolk sac is connected to the digestive tube through the omphalomesenteric (vitteline) duct. The dorsal mesentery separates the digestive tube from the dorsal wall of the embryo, and at this stage there is also a ventral mesentery that separates the anterior part from the ventral embryonic wall. Continuity with the exterior environment is formed only after the rupture of the buccopharyngeal and cloacal membranes. The anatomic formation of the esophagus, stomach, intestine, pancreas and liver is achieved by the fourth week through a series of evaginations, elongations and dilatations. Further development through cell proliferation, growth and morphogenesis then follows.

The pharynx, esophagus, stomach, liver, gallbladder, pancreas and upper duodenum originate from the foregut. The distal portion of the duodenum, the jejunum, ileum, cecum, appendix, ascending colon and two-thirds of the transverse colon arise from the midgut. The hindgut differentiates into the third distal part of the transverse colon, the descending colon, the sigmoid colon, the upper two-thirds of the rectum and the urogenital sinus [1]. Arterial supply delineates the boundaries: the celiac axis supplies the foregut, and the superior and inferior mesenteric arteries supply the midgut and hindgut, respectively.

The rapid elongation of the gut during the first trimester leads to its herniation into the umbilical cord. The gut reenters into the abdominal cavity and rotates counterclockwise around the superior mesenteric artery to reach its final position by 20 weeks of gestation (Fig. 42.1). Failure of this process results in malrotation.

By the second trimester all the anatomic structures of the gastrointestinal tract are well formed and recognizable. The process of maturation involves rapid increase in the mucosal surface area followed by folding forming the villi and microvilli. However, functional maturation, including the appearance of digestive enzymes in the villi and the development of swallowing and mature motility patterns, occurs much later than structural development; some functions are not fully established until 2-4 years of age. Maturation tends to follow a cephalocaudal progression from the proximal to the distal part of the gastrointestinal tract (Table 42.1).

The regulation of timing and nature of growth and maturation of the gastrointestinal tract is complex and involves many factors. These include not only intrinsic factors like signals arising from gene expression [2], biologic clock or regulatory hormones and peptide growth factors, but also extrinsic environmental factors acting at the molecular level. The gastrointestinal tract is in continuity with the environment; the fetal gut is thus exposed to the amniotic fluid content as the neonatal gut is exposed to enteral feedings, nutrients and even microbes. This is related to the advantages of breast milk with its immunologic and trophic growth factors to the newborn [3]. At the cellular level regulation occurs by homeodomain transcription factor genes [1] and cell to cell interactions, especially epithelial-mesenchymal interactions [4]. The pluripotent stem cell located in the crypt of the villus continuously differentiates into the 4 gut cell lineages: absorptive enterocytes, mucous (goblet), Paneth and endocrine cells [5]. This process that involves continuous proliferation, migration and loss of epithelial cells along the mucosal surface is also regulated genetically by homeodomain transcription factors [1].

For detailed aspects on structural anatomic development of the different parts and organs along the gastrointestinal tract, refer to Chapter 90, where congenital malformations of the gastrointestinal tract are discussed.

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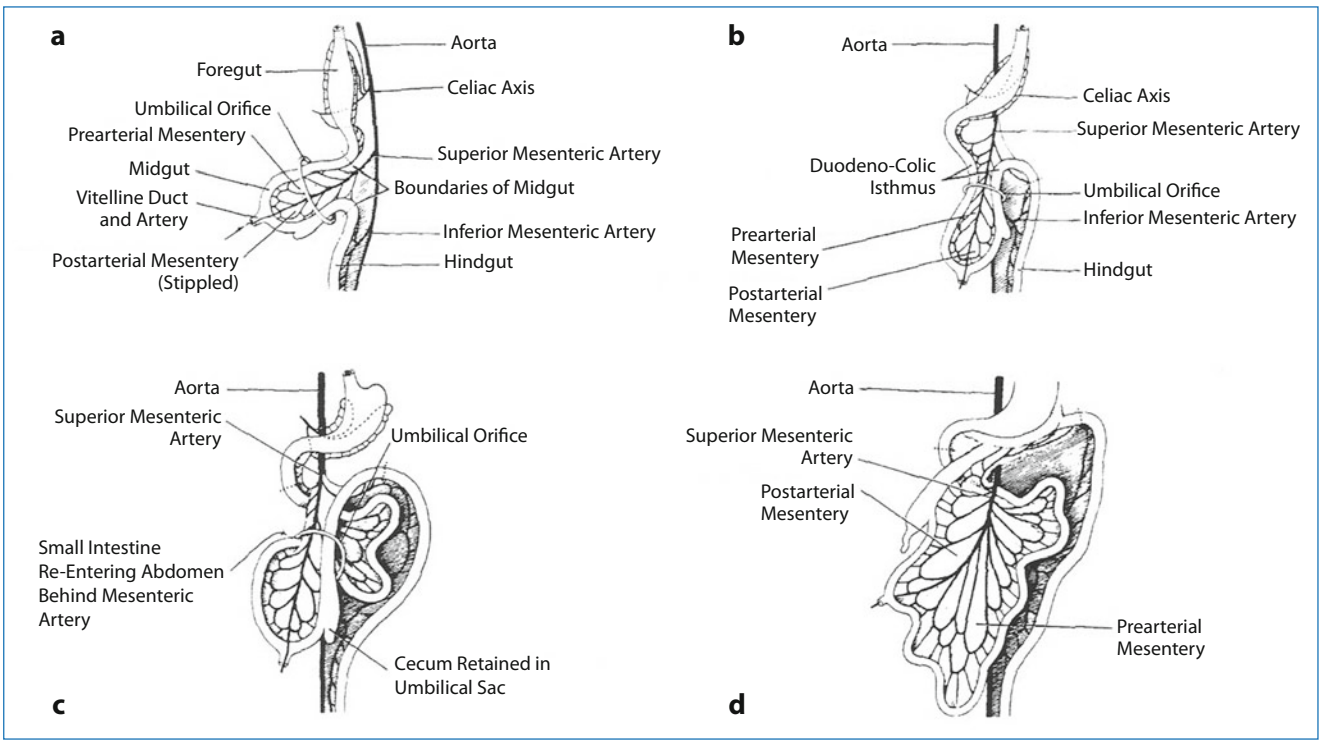

Fig. 42.1 Diagram showing normal rotation of alimentary tract. **a** Fifth week of intrauterine life (*lateral view*). The foregut, midgut, and hindgut are shown with their individual blood supply supported by the common dorsal mesentery in the sagittal plane. The midgut loop has been extruded into the umbilical cord. **b** Eighth week of intrauterine life (*anteroposterior view*). The first stage of rotation is being completed. Note the narrow duodenocolic isthmus from which the midgut loop depends and the right-sided position of the small intestine and left-sided position of the colon. Maintenance of this position within the abdomen after birth is termed *nonrotation*. **c** About the 10th week of intrauterine life, during the second stage of rotation (*anteroposterior view*). The bowel in the temporary umbilical hernia is in the process of reduction; the most proximal part of the prearterial segment entering the abdomen to the right of the superior mesenteric artery is held forward close to the cecum and ascending colon, permitting the bowel to pass under it. As the coils of small intestine collect within the abdomen, the hindgut is displaced to the left and upward. **d** Eleventh week of intrauterine life at the end of the second stage of rotation. From its original sagittal position, the midgut has rotated 270 degrees in a counterclockwise direction about the origin of the superior mesenteric artery. The essentials of the permanent disposition of the viscera have been attained. Reproduced with permission from: Gardner CE Jr, Hart D, Anomalies of intestinal rotation as a cause of intestinal obstruction. Arch Surg 1934;29:942–981. Copyright 1934 American Medical Association

Table 42.1 Anatomic and functional maturation of the gastrointestinal tract

Postconceptional age (weeks)					
15	20	25	30	35	40
Mouth	Salivary glands	Swallow	Lingual lipase	*Sucking*	
Esophagus	Muscle layers present	Striated epithelium present	*Poor lower esophageal sphincter tone*		
Stomach	Gastric glands present	G cells appear	Gastric secretions present	*Slow gastric emptying*	*
Pancreas	Exocrine and endocrine tissue differentiate	Zymogen present	*Reduced trypsin lipase*		*
Liver	Lobules form	Bile secreted	*Fatty acids absorbed*		*
Intestine	Crypt and villus form	*Glucose transport present*	*Dipeptidase, sucrase, and maltase active*	*Lactase active*	
Colon		Crypts and villi recede		*Meconium passed*	

* Full functional maturation occurs postnatally.
Italics indicate functional maturation.
Reproduced with permission from Berseth, CL. Developmental anatomy and physiology of the gastrointestinal tract. In: Taeusch HW, Ballard RA (eds) Avery's diseases of the newborn, 8th edn. W.B. Saunders Company, Philadelphia, Copyright W.B. Saunders.

42.2 Vascularization

Vascular supply to the intestine develops concurrently with its anatomic development. The arterial supply develops as 3 ventral outbuddings of vessels from the aorta: celiac trunk supplies the stomach, pancreas and duodenum via its splenic, left gastric and hepatic branches; superior mesenteric artery supplies the jejunum and ileum; inferior mesenteric artery supplies the colon and rectum. Smaller branches of the superior mesenteric artery form arcades before entering the intestinal wall that along with anastomoses with branches of the inferior mesenteric artery provide rich collateral flow. Watershed areas of marginal blood supply however exist in the distal transverse colon and upper rectum. Since there is high metabolic activity in the intestinal mucosal layer, it has rich vascular supply, yet it is still the most sensitive layer to any derangements in blood supply.

Based on animal studies it is assumed that intestinal basal vascular resistance decrease in the immediate postnatal period, thus doubling blood flow and oxygen delivery in the neonate to compensate for the higher metabolic activity related to nutrient absorption that was not needed in fetal life. However, later on between postnatal days 12–30 vascular resistance increases again due to maturation of the intestinal extrinsic adrenergic innervation, as well as changes in the geometry of the intestinal vasculature in response to the rapid increase in size (length and surface area) of the gut that happens concomitantly [6].

Basal vascular resistance is mediated by nitric oxide (NO) that is continuously produced by the endothelial cell NO synthase isoform (ecNOS) or is increased in response to mechanical or chemical stimuli. NO causes vasodilatation of the adjacent vascular smooth muscle via production of cGMP by activated guanylate cyclase. Rates of NO production and its relaxing effect on the mesenteric artery bed are maximal immediately after birth. Its role in determining vascular tone lessens over the first month of life. The essential role of endothelial production of NO in maintaining newborn intestinal hemodynamics may be important in the pathogenesis of necrotizing enterocolitis (NEC), because endothelial dysfunction that would limit NO production may lead to vasoconstriction and substantial intestinal ischemia [6].

Individual vascular smooth muscle cells respond to stretch stimulus by contracting via stimulation of calcium-induced phosphorylation of myosin light chain kinase [7]. This intrinsic myogenic response is the second mechanism mediating vascular response in the neonatal intestine, and it has an important role in setting basal intestinal vascular resistance in the first days of life [6]. It causes vasoconstriction in response to increases in intravascular pressure in some of the blood vessels.

Endothelin, the third mediator of vascular resistance, is a vasoactive peptide mainly produced by the vascular endothelium. ET-1, the intestinal form, is continuously produced by the intestinal vascular endothelium in an age-specific manner. Its production is greater in young subjects, especially in newborns, probably reflecting its essential role in angiogenesis at this time of exceptionally rapid growth of the intestine. Endothelin binding to ET_A receptors on vascular smooth muscle induces potent sustained contraction, while its binding to ET_B receptors causes NO-mediated vasodilatation. The force of ET_A-induced vasoconstriction exceeds the modest ET_B-induced NO-dependent vasodilatation, thus the net effect of constitutive ET-1 production is vasoconstriction [8].

The significance of these three mechanisms regulating intestinal vascular resistance in the neonate is markedly diminished after the first postnatal month of life. Thereafter, the greater degree of extrinsic adrenergic innervation causes increased basal vascular resistance with more robust responses to baro- and chemo-reflexes.

Mesenteric blood flow is regulated by changes in the tone of the arteriole and the pre-capillary sphincter. Control of blood flow is intrinsic and extrinsic [6]. Intrinsic control is achieved by local factors that react to changes in arterial pressure (pressure-flow autoregulation) and tissue oxygenation, such as functional postprandial hyperemia in response to feeding or reactive hyperemia in response to vessel occlusion. Modest arterial hypoxemia (PO_2 around 50 mmHg) causes vasodilatation and increased perfusion to the gut, yet profound hypoxemia (PO_2 <40 mmHg) causes vasoconstriction and gut ischemia. These different responses probably reflect NO regulation of vascular tone [6]. Extrinsic regulation is mediated by sympathetic input from the splanchnic nerves. However, the vasoconstriction response to adrenergic nerve stimulation is short-lived, and gut blood flow is slowly restored to its baseline by a process termed autoregulatory escape.

Circulating endogenous and exogenous factors (e.g., hormones, histamine and prostaglandins) can also modulate vascular tone. The presence of nutrients in the gut triggers the postprandial hyperemic response with brisk vasodilatation and increased blood flow and oxygen delivery. Postprandial hyperemia in the newborn is mediated by dilatation of the intestinal circulation in response to substance P, which is a peptide neurotransmitter in the enteric nervous system [9]. Both postprandial hyperemia and autoregulatory escape phenomena are present in newborns. However, unlike term infants, healthy preterm infants require compensatory systemic hemodynamic changes in response to feeding and postprandial hyperemic response because the increased mesenteric flow causes cardiac output increase and systemic blood pressure decrease [10]. Drugs used in neonatal care such as indomethacin [11], dopamine and caffeine [12], as well as other treatments (e.g., hyper alimentation and CPAP) [13] may diminish intestinal mesenteric blood flow thus disrupting compensatory mechanisms.

42.3 Neural Control of Motor Function and Gastrointestinal Motility

A major function of the gut is motor activity that moves the content forward along the gastrointestinal tract. This includes sucking, swallowing, gastric emptying, propagation of the food along the small intestine and evacuation of wastes from the colon. Neural crest cells migrate into the developing gut, and with muscle cells differentiate to form three layers of muscle that surround the mucosa (by 14 weeks of gestation) and a neural network that controls motor activity (by 20-24 weeks of gestation). This neural network comprises the enteric nervous system (ENS). The phasic contractions of the gastrointestinal tract are regulated by a complex interplay between the myogenic, neural and chemical control mechanisms [14].

Peristaltic activity allows intestinal contents to move along the intestine and can occur through a variety of mechanisms. Phasic contractions are responsible for the mixing and propulsive movements of the gut after a meal. These are called migrating action potential complexes (MAPCs), and they are prominent postprandially [14, 15]. During fasting, organized groups of contractions called cyclic motor activity and migrating motor complex (MMC) keep the upper digestive tract clean of residual food and debris [14, 15]. MMC cyclic motor contractions originate from the antrum and progress to the ileum. They are characterized by periods of quiescence followed by irregular contractions, then a period of regular contractions and return to the quiescent state. In addition, the small intestine and the colon generate giant migrating contractions, which are several-fold stronger than the postprandial phasic contractions and migrate uninterrupted over long distances. The giant migrating contractions are effective in rapid propulsion. The upper small intestine and the gastric antrum may also generate retrograde giant contractions that generally precede vomiting [14]. Movements of MMCs and MAPCs are orchestrated by the ENS.

42.3.1 Myogenic Control

Contractile activity can also propagate toward the anus in association with propagating slow-wave activity [16], expressing myogenic control. Muscle cells generate this spontaneous electrical activity through fluctuations in resting membrane potential, and these periodic depolarizations are called slow waves, electrical control activity, basic electrical rhythm or pacemaker activity. This myogenic pacemaker slow wave activity originates from specialized smooth muscle cells, the interstitial cells of Cajal (ICC) [17], that form an electrical syncytium with the smooth muscle layers. The network of ICC is also associated with the Auerbach's plexus [18]. There is evidence that absence of an ICC network may contribute to abnormalities in intestinal transit in humans, including infantile hypertrophic pyloric stenosis and Hirschsprung's disease [19, 20]. The fact that ICC are always very intimately associated with neural structures suggests a close co-operation between myogenic and neural control. Contractions occur when a slow wave under the influence of a neural or chemical stimulation exceeds the excitation threshold necessary for an action potential called electrical response activity or spike potential. Following this depolarization of the smooth muscle cell, intracellular calcium levels increase, calcium binds to the regulatory protein calmodulin, and this permits the binding of the contractile proteins actin and myosin, resulting in a contraction. It can be seen that the contraction caused by the electrical response activity occurs on the background of the electrical control activity, i.e., the slow waves of myogenic control [21]. This background slow wave activity controls timing, speed and direction of the intestinal contractions. There is probably co-operation between the neural and myogenic control of intestinal motility to ensure proper transit [15]. Denervated gut muscle cells have therefore a basal contraction rate like cardiac muscle cells.

42.3.2 Enteral Nervous System

The ENS is a subsystem of the autonomic system that includes two major nerve plexuses situated in the bowel wall. The myenteric (Aurbach) plexus lies between the external circular and longitudinal muscle layers, and the submucosal (Meissner) plexus lies between the circular muscle layer and the muscularis mucosa. The ENS also includes six minor plexuses located in the different layers of the bowel wall (Fig. 42.2). The ENS is integrated within the central nervous

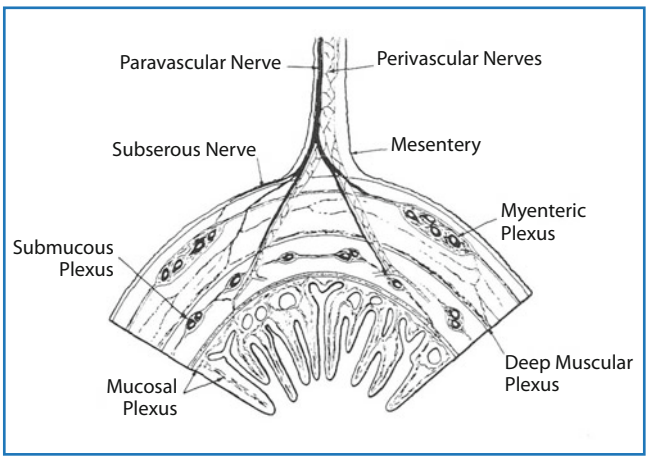

Fig. 42.2 Arrangement of enteric plexuses in whole mounts of intestine. In addition to the large myenteric and submucosal plexuses, several smaller plexuses are shown. Reproduced with permission of Elsevier from: Furness JB, Costa M, Arrangement of enteric plexuses. In: Furness JB, Costa M (eds) The Enteric Nervous System. Churchill Livingstone, New York, 1987

system (CNS) control of the gut, but it also provides the final regulation, and is even able to regulate gut motility, secretion and vascular flow independent of the CNS and spinal nervous control. The ENS with its large number of neurons compared to the relatively sparse central innervation of the gut is thus termed the "little brain of the gut", stressing its uniqueness compared to other peripheral nerve systems [22].

The basic unit of the ENS is the nerve cell. Neurotransmitters are released to other neurons or directly act upon striated or smooth muscle cells. Acetyl-choline is the main excitatory neurotransmitter mediating smooth muscle contractions [23]. Glutamate is also an excitatory neurotransmitter acting on N-methyl-D-aspartate (NMDA)-type receptors in the myenteric plexus in conjunction with cholinergic stimuli [24]. Glutamate can be synthesized from exogenous L-glutamine in myenteric neurons, and be released in response to neuronal depolarization [24]. Norepinephrine is a main inhibitory neurotransmitter. Nitric oxide (NO) mediates non-adrenergic non-cholinergic (NANC) relaxation of gastrointestinal smooth muscle [25]. NO synthase (NOS) and NO-containing enteric neurons have been demonstrated in the ENS [23]. Vasoactive intestinal peptide (VIP), another inhibitory neurotransmitter, may mediate NO effects [26]. Absence of NO has been implicated in the pathogenesis of infantile hypertrophic pyloric stenosis [27] and Hirschsprung disease [28].

Enteric neurons and glial cells of the ENS originate from the neural crest [29]. Cells from the vagal region migrate to the whole length of the gastrointestinal tract, but cells from the truncal and sacral regions populate only the foregut and the post-umbilical hindgut, respectively [30]. Like intestinal smooth muscle development, neural cells from the vagal region develop in a cranio-caudal direction, as opposed to neural cells originating from the sacral region that migrate into the distal bowel in a caudo-cranial direction. Neural cells that migrate from the outer mesenchyme of the gut to the inner mucosa proliferate. At this stage the cells are pluripotent and transiently express catecholamines [31]. Later in gestation the neural cells in the gut wall mature and differentiate into enteral neurons that do not express catecholamines anymore. Differentiated neurons express serotoninergic and peptidergic (e.g., substance P and neuropeptide Y) neurotransmitters. Some of the neural crest-derived cells that colonize the fetal bowel cluster and form ganglion cells that form the two ganglionated plexuses, described above [32]. These ganglion cells are distributed normally by 24 weeks gestation, although their density continues to change over the first years of life [33]. In the human fetus by the 10th week of gestation the circular muscle layer is formed, followed by the appearance of a primitive myenteric plexus, and the longitudinal smooth muscle layer in the 12th week of gestation. Most neurons in the myenteric ganglia at the 10th week of gestation are undifferentiated neuroblasts. The time between the 10th and 18th week of gestation is important for both morphological and functional maturation of the ENS. In the 18-week-old human fetus most neurons in the myenteric ganglia are already differentiated and mature, and the organization of smooth muscle cells and the primary strands of the myenteric plexus provide a satisfactory basis for an integrated peristaltic movement that is already seen in the gut [34]. Neurotransmitters are evident by 24-26 weeks, although their distribution along the gut may change and reach adult pattern only close to term.

Abnormalities in migration and differentiation of neural crest cells may cause a number of gastrointestinal disorders [35, 36], the main one is Hirschsprung disease that results from the absence of enteric ganglia in the distal hindgut.

42.3.3 Central Nervous System Control

CNS and ENS that control gastrointestinal motility, secretion and vascular flow consist of afferent sensory neurons, efferent motor neurons, synapses to the effector (e.g., muscle) cells and interneurons that integrate the messages. Sensory afferent fibers travel with parasympathetic preganglionic fibers in the vagus nerve and sympathetic postganglionic fibers in the splanchnic nerves. Vagal afferents conduct impulses from the mucosal and muscular layers of the whole gut, from the soft palate to the distal colon [37]. They reach the liver, gallbladder and pancreas too. Splanchnic afferents innervate the mucosal and muscular layers, the serosa and the bowel mesenterium. Motor innervation is made of sympathetic and parasympathetic components of the autonomic nervous system. Preganglionic sympathetic neurons are found in segments T2-L2 of the spinal cord. Postganglionic sympathetic neurons are found in the pre-vertebral sympathetic trunk (celiac, superior and inferior mesenteric ganglia). Preganglionic parasympathetic neurons are present in the medulla and in the sacral region of the spinal cord. Postganglionic parasympathetic neurons are located within the gastrointestinal tract. Most of the parasympathetic innervation of the gut comes from the vagus. Vagal efferents end up in the enteric ganglia of the ENS [38]. Sacral nerves innervate only the distal colon from the middle of the transverse colon. Since the vagus innervates most of the gut and has both afferent and efferent fibers it needs precise central viscerotopic organization of inputs and outputs. Afferent fibers reach the nucleus tractus solitarius and the vagal sensory nucleus. Efferent fibers originate from the motor nuclei and dorsal motor nucleus of the vagus and the nucleus ambiguous [37, 39]. The nucleus tractus solitarius is an important synaptic site where interneurons coordinate afferent inputs with efferent outputs delivered to the nucleus ambiguous and the dorsal motor nucleus of the vagus in order to control esophageal and gastrointestinal motility. Glutamate, an excitatory amino acid, is considered one of the neurotransmitters involved in this brain-stem synaptic transmission via NMDA receptors, specifically involved in esophageal motor activity in response to swallowing [40].

42.3.4 Gastrointestinal Motility

The motor activity of the gastrointestinal tract is critical not only for feeding but also for digestion and absorption of nutrients, because it promotes mixing of the nutrients with pancreatic, biliary and intestinal secretions, and transit of the intestinal content forward. Smooth muscles of the intestine are arranged in outer longitudinal and inner circular layers. They develop in a cranio-caudal direction that resembles the development of the vagal neural crest derived ENS. By the end of the first trimester longitudinal and circular muscles are detectable in the ileum. During the rest of gestation, muscle thickness increases. By 28 weeks gestation contraction of the gut can produce 60% of the intraluminal pressures measured at term. These pressures are sufficient for preterm infants to be able to move intestinal content forward. There is a gradual maturation of gut motility during fetal life that continues in the first years of postnatal life [41]. The gradual maturation of gut motility is the result of muscular and neural maturation. Motor activity is immature in preterm infants. Normal propulsive motility of the gut is evident by 30 weeks gestation. The classical MMC (migrating motor complexes) are demonstrated by 33 weeks but at slower propagation rates and without being abolished by feeding as happens in older children and adults.

Sucking and swallowing involve skeletal and smooth muscles. Although sucking and swallowing reflexes appear early in gestation their maturation is not complete until after birth. Fetuses can swallow amniotic fluid by 15-17 weeks gestation. Exposure to swallowed amniotic fluid including its growth factors such as the epidermal growth factor (EGF) is important for gastrointestinal development [42]. Close to term the fetus swallows 450-750 mL of amniotic fluid each day. Esophageal or intestinal atresia results in polyhydramnios. Non-nutritive sucking appears between 18-20 weeks, but nutritive sucking that involves coordinated mechanisms of sucking, swallowing and breathing develop only by 34-35 weeks of gestation [43], but has been described earlier (as early as 30-32 weeks) in preterm infants after early oromotor stimulation of nonnutritive sucking and use of pacifiers in preterm infants [44]. The maturation of nutritive sucking is parallel to the rapid growth in gastric size and maturation of gastric emptying, gastric antral and small intestinal motility and stimulation of secretion of gastrointestinal enzymes and peptides that enhance weight gain as well. Early introduction of oral feeding to preterm infants accelerates maturation of nutritive sucking and the transition time from tube to full oral feeding [44]. Maturation of sucking skills correlates better with postmenstrual gestational age than with postnatal age in preterm infants [45]. Lau et al characterized five developmental stages of sucking in preterm infants that were found to be in correlation with postmenstrual age, feeding progress, and the number of daily oral feedings. Rythmicity of sucking and rate of transfer of milk into the posterior pharynx were enhanced when infants reached the more mature stages of sucking, and general oral feeding performance improved as infants' sucking skills matured [46]. The first stage of swallowing is an involuntary reflex in preterm and term infants induced by the presence of milk in the posterior pharynx. At term, sucking is followed in an orderly manner by swallowing, esophageal peristalsis, relaxation of the lower esophageal sphincter (LES) and relaxation of the gastric fundus.

The esophagus serves as a connecting tube between the oropharynx and the stomach, but its peristaltic activity is essential for adequate passage of nutrients and for adequate clearing of refluxed materials. Coordinated esophageal peristalsis is present by 32 weeks of gestation and by that time upper esophageal sphincter (UES) tone is already present. Esophageal sphincters are not anatomic but rather functional. The LES is an area of increased muscular tone in the distal esophagus. Its main functions are to prevent gastric acid reflux and to propel food into the stomach. In term infants LES tone is 20-40 mmHg. However, contraction amplitudes, propagation of pressure waves and LES tone are lower in preterm compared to term infants. LES tone in a preterm infant less than 29 weeks gestation is lower than 5 mmHg [47]. Gastroesophageal reflux (GER), which is more common in preterm infants, is probably related to low LES tone and poor regulation of its relaxation [48]. Using impedance pH monitoring, non-acid as well as acid GER has been demonstrated [49], though norms for premature as well as term infants are yet to be established . The esophagus also provides aerodigestive defenses that exist in the healthy term infant and prevents aspiration of bolus feed or secretions. These mechanisms include: basal UES and LES tonic contraction, primary persitalsis of the esophagus triggered by swallowing, and secondary peristalsis with increase in UES tone in response to esophageal provocation. These defenses may not function in infants with neurodevelopmental abnormalities or mal-development of the foregut. Also, these defenses may not be fully mature in preterm infants, especially those with chronic lung disease [50].

Gastric emptying of liquid feeds is regulated by the fundus and proximal third of the stomach, which dilate to accommodate the milk volume, and is dependent on the pressure gradient between the proximal stomach and the duodenum. Accommodation of large volumes of milk in the fundus is poor immediately after birth. During the first three postnatal days, the newborn stomach becomes more compliant and develops more receptive relaxation, associated with a larger volume capacity. This is in accordance with the small feedings that neonates ingest in the first days of life [51]. Gastric emptying of more solid contents is regulated by the distal portion of the body of the stomach, the antrum and the pylorus where mixing is taking place. Gastric emptying requires adequate and coordinated antral and duodenal motor activities. As opposed to antral motor activity that seems to be mature as early as 24 weeks gestation [52], the coordination and the level of duodenal motor activity are decreased in preterm infants. In half of the preterm infants duodenal contractions cease in response to feeding resulting in delayed gastric emptying [53]. Reduced

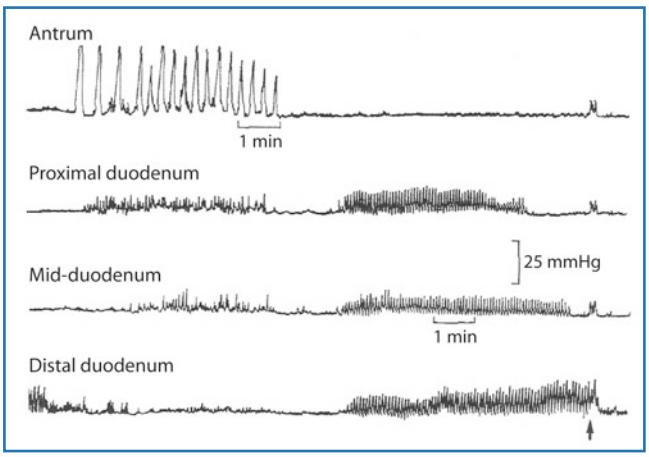

Fig. 42.3 Migrating motor complex in a term infant. Upper tracing: motor contractions recorded from the antrum. Lower three tracings: motor contractions recorded from the duodenum. Phasic activity present in the antrum is temporally associated with the appearance of intense phasic activity that migrates distally to the three duodenal leads. Reproduced with permission from: Ittman PI, Amarnath R, Berseth CL, Maturation of antroduodenal motor activity in preterm and term infants. Dig Dis Sci 1992;37:14–19

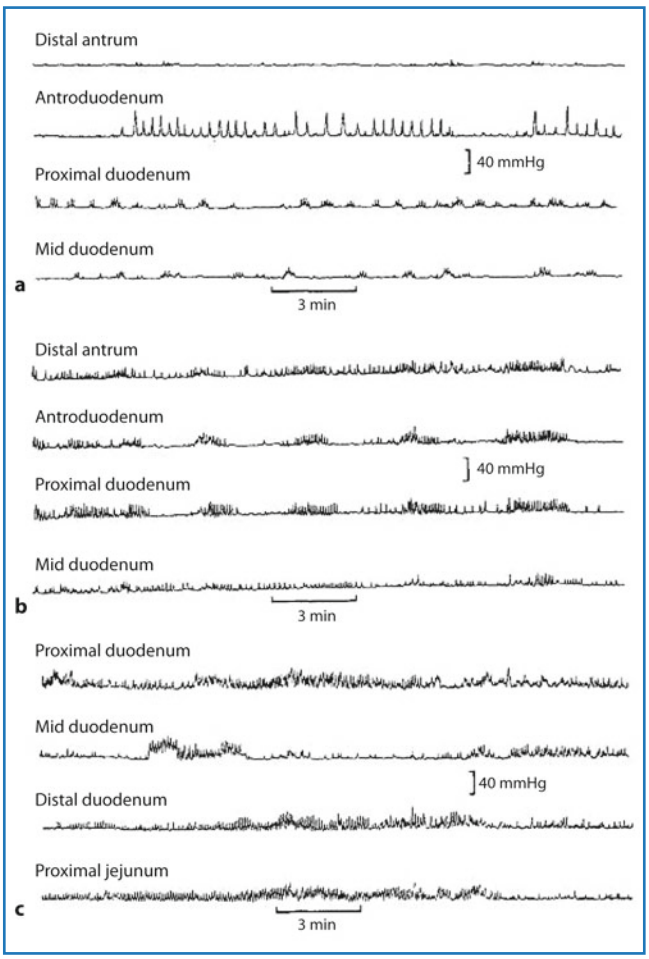

Fig. 42.4 Representative serial tracings from an individual subject, at postconceptional age of 32 weeks (**a**), 34 weeks (**b**) and 36 weeks (**c**), demonstrating increasing cluster duration and decreasing cluster frequency. Reproduced with permission from: Berseth CL, Gestational evolution of small intestine motility in preterm and term infants. J Pediatr 1989;115:646–651. Copyright 1989 Elsevier

osmolality and increased feeding volumes seem to increase gastric emptying, but gastric emptying remains strongly related to gestational age at birth even at age of one month [54].

Maturation of gastrointestinal motility with increasing gestational age is most evident in the small intestine. This is shown by longer transit times as infants are more immature, especially before 30 weeks when ineffective gut motility may interfere with enteral feeding. The Migratory Motor Complexes (MMCs), described above, act as "housekeepers" that propel luminal content caudally along the small intestine. Term and late preterm infants born after 36 weeks gestation present organized MMC pattern [52] with periods of quiescence followed by irregular contractions, then regular contractions and return to the quiescent baseline (Fig. 42.3). This pattern is rarely found in preterm infants. Between 24-28 weeks gestation only unorganized irregular contractions are recorded with few quiescence intervals [41, 52]. By 28-32 weeks periods of quiescence appear alternating with short bursts of phasic activity called clusters. Between 32-36 weeks motor patterns become more organized and the periods of quiescence and clusters lengthen. MMCs are first seen around 33 weeks (Fig. 42.4) [41, 52]. Feeding should normally disrupt MMC pattern by creating strong contractions originating from the gastric antrum and pylorus that mix gastric content and propagate it through the pylorus and along the small intestine. These contractions are the migrating action potential complexes (MAPCs), discussed above. Berseth suggested that early feeding may accelerate the maturation of gut motility in preterm infants [55]. Composition and caloric content of the food, but not its volume, also has an effect on the maturation and intensity of the gut motor response, suggesting that diluted formula may not provide an optimal stimulant for the preterm intestinal functional responses to feeding [56].

94% of term newborn infants pass meconium within the first 24 hours and close to 100% do so within the first 48 hours. Passage of meconium may be delayed in preterm infants, up to 10 days and more in the very-low-birth-weight preterm infants [57]. Normal colonic function is necessary for successful defecation. However, not many studies were done on colonic motility, especially in preterm infants. The colon transports non-absorbable substances for excretion and facilitates exchange of water and electrolytes. Transit is slow and is achieved by mass movements that sweep the colon. Colonic motility is characterized by irregular alterations of quiescence with non-propagating and propagating contractions [58]. Colonic propagated events are defined as low and high-amplitude propagated contractions (LAPC and HAPC), and HAPCs are responsible for the mass movements. A colonic motor

response to a meal is comprised of segmental contractions, increased colonic smooth muscle tone and possibly also HAPCs. These were described in children as well, HAPCs being more frequent [59]. The only colonic cyclic activity is distal to the rectosigmoid junction. It is called recto motor complex and it is not synchronous with the small bowel MMCs. It keeps the rectum empty, especially at night, and thus helps maintaining continence [60]. It seems that colonic motility in term neonates is similar to that found in older children. However, colonic motility is immature in preterm infants. This is supported by the fact that term newborns that develop hypoxemia pass meconium in utero, while preterm infants rarely do so, suggesting the production of contractions that create mass stool movements are immature in preterm babies. Hirschsprung disease is the most common motility disorder affecting the colon. Neuronal Intestinal Dysplasia (NID) of the colonic submucous plexus is considered a congenital malformation of the enteric nervous system causing symptoms resembling those of Hirschsprung's disease. It is a poorly understood colonic motility disorder that is often associated with Hirschsprung's disease and is also associated with chronic intestinal pseudo-obstruction, anorectal malformations and the Multiple Endocrine Neoplasia (MEN) II syndrome [61]. Anal sphincter function is mature in term infants, but may be immature in less than 30 weeks gestation premature infants. However, delayed meconium passage in preterm infants could not have been related to the absence of a recto anal inhibitory reflex [62].

42.4 Digestion and Absorption

Three steps can be defined in the process of nutrient absorption: digestion, which is the processing of nutrients within the intestinal lumen and at the terminal digestive sites of the brush border membrane of the mucosal epithelial cells; absorption in the epithelial absorptive surfaces, which involves the membrane transport systems of the small intestinal epithelium; and transport of nutrients into the circulation [63].

42.4.1 Fat Digestion and Absorption

Fat is the major source for energy (provides half of the caloric intake) [64] and polyunsaturated fatty acids in newborns [65]. It is an essential cell membrane component [65], and it facilitates the absorption of fat soluble vitamins. Most of the lipids are absorbed in the proximal two thirds of the jejunum.

Digestion and transport of fat in the lumen of the gastrointestinal tract requires solubilization of the dietary lipids, mostly triglycerides, in the aqueous medium of the gut content, i.e., emulsification. This involves mechanical actions of mastication and gastric mixing to form fat droplets, as well as coating by phospholipids (mostly phosphatidylcholine in food with the addition of bile phospholipids in the duodenum) to stabilize the emulsion. Emulsification allows exposure of a large surface area to the action of the lipolytic enzymes. Hydrolysis of fat to form mono- or diglycerides and free fatty acids begins, especially in newborns, in the stomach by lingual lipase secreted from Ebner's glands near the circumvallate papillae [66] and by gastric lipase secreted by gastric glands. Lingual and gastric lipases are essential for the digestion of milk fat in the newborns because they can penetrate into the milk fat globule and initiate the digestive process, contrary to the pancreatic or milk digestive lipases [67]. In newborns, lingual lipase contributes significantly to lipid hydrolysis, and non-nutritive sucking stimulates its release from the posterior part of the tongue. Lingual and gastric lipases that are present as early as 26 weeks gestation compensate for the limited action of bile acids and pancreatic lipases that are found in low amounts, especially in preterm infants [68]. The contribution of lingual lipase significantly declines later in life.

Gastric hydrogen ions enter the duodenum and stimulate the release of secretin that enhances bicarbonate secretion from the pancreas. This process that raises the intraluminal pH to 6.5, which is more suitable for further fat digestion, is less effective in newborns. The optimum pH of lingual lipase, which is 3.5-6.0, is compatible with its continued activity in the upper small intestine, especially in newborns, where the luminal pH is under 6.5 [68]. The initial gastric phase of lipolysis generates modest amounts of diglycerides, monoglycerides, and free fatty acids that are required with bile salts for optimal activity of the intestinal phase of lipolysis. The bile salts further enhance fat emulsification rendering it more susceptible to the action pancreatic lipase that degrades it to monoglycerides and fatty acids. The co-activity of the enzyme colipase is critical to facilitate its attachment to the triglyceride droplets and to prevent bile salts from deactivating pancreatic lipase. All pancreatic lipases levels are low after birth, especially in preterm low birthweight infants [68], and increase to adult levels only by six months of age. The colipase-dependent pancreatic lipase catalyzes the intraduodenal phase of triglyceride digestion in formula-fed infants. However, in breast-fed infants this process is also mediated by the bile salt-stimulated lipase (BSSL), the main lipase in breast milk that is capable of hydrolyzing all three ester bonds of triglycerides, thus shifting the final products of triglyceride digestion to glycerol and free fatty acid that promote efficient fat absorption [69]. BSSL is identical to the pancreatic bile salt stimulated lipase that is responsible also for hydrolyzing intestinal cholesteryl esters and phospholipids [70], and its presence in human milk may serve as a bridge until the pancreas secretes it in adequate amounts. BSSL is not destroyed and does not lose its activity in the stomach, thus it can reach the duodenum and be activated by the concentrations of bile acids as found in the infant's duodenum. Breast milk also contains lipoprotein lipase (LPL) that has been described as related to the

prolonged neonatal jaundice in breast-fed infants. Lingual, gastric and milk lipases compensate for the low pancreatic lipase activity in term and preterm newborns [68], and since they all function well also in the presence of low bile acids concentrations they can also function well in preterm infants where bile acids synthesis in the liver is decreased and their intraluminal concentration is half of the critical micellar concentration required for fat absorption [68].

The source of dietary fat influences absorption [71], human milk being the best source for term and preterm newborns [72]. Unsaturated fatty acids are absorbed better than saturated ones, and short- medium-chain triglycerides are absorbed better than long-chain triglycerides [71]. Yet, long-chain fatty acids (≥16C) may have a growth promoting effect on intestinal cells, as can be seen in short-bowel syndrome [73]. Intraluminal calcium concentration also influences fat absorption. High calcium intake, as found in cow's milk, can impair fat absorption in term and preterm newborns. In human milk, palmitic acid esterified at the sn-2 position of the triacylglycerol molecule is well absorbed as 2-monopalmitin [74]. Lower digestibility of fat in bovine milk based infant formulas compared to human milk is related to the different position of palmitic acid in both fat sources [75].

After the luminal phase of digestion and processing of the lipids is completed, the mixture of all lipolytic products in the lumen is mixed with bile salts to form small aggregates of mixed micelles or liposomes, which are larger aggregates that are ready for the next mucosal phase of absorption. The bile salts that have both lipid- and water-soluble domains allow the passage of micelles through the water layer at the mucosal cell surface and the lipid rich microvillus membrane. The bile salts that are not absorbed remain in the intestinal lumen and are actively reabsorbed at the terminal ileum and into the portal system to be re-secreted into the bile. This enterohepatic circulation plays an important role in the pathogenesis of neonatal hyperbilirubinemia. Monoglycerides and free fatty acids diffuse into the cells. A significant proportion of this phase of lipid absorption is not by passive diffusion, but involves active, protein-mediated, transport [76, 77]. The major fatty acid protein transporter so far identified is FATP4 that is mainly involved in long-chain fatty acids absorption [78]. These fatty acids transporter proteins are located on the apical side of the enterocyte [76]. Inside the absorptive cells of the villus fatty acids are transported to the smooth endoplasmic reticulum where the triglycerides are re-synthesized. The triglycerides along with cholesteryl esters, phospholipids and apoproteins are packed together as chylomicrons that bind to the basolateral membrane and are transported via the intestinal lymphatics into the circulation [77]. Fat absorption seems to be fully developed at birth to suit the high fat intake of newborn infants [64]. This seems to be true for preterm infants too, thus suggesting that the relative inefficiency of fat absorption in preterm infants (40-90%) is mostly attributed to immaturity of the intraluminal digestive mechanisms, as described above.

42.4.2 Protein Digestion and Absorption

Although proteins encompass 10-15% of the caloric intake, they are important for somatic growth and synthesis of intracellular structures and enzymes. Adequate protein intake is critical for the premature infant, especially for brain development [79]. The initial step of protein digestion takes place in the stomach, and includes denaturation by gastric acidity and proteolysis mostly into large polypeptides by gastric pepsins. Pepsinogen 1 and 2 are the pro-enzymes of pepsin that are activated in low pH. Proteolysis in the stomach is affected by gastric content and motility and most importantly by gastric acidity. The pH of gastric juice is neutral or slightly acid at birth and decreases within hours. Gastric acid flow doubles within the next two months [80]. Parietal cell activity is noted in the body, antrum and pyloric regions of the fetal stomach as early as 13 weeks [81]. However, although the fetus has the capacity to produce gastric acid from the middle of the second trimester, gastric acid secretion is significantly reduced in preterm infants [82], and increases with growing postnatal and gestational age. Parietal cells disappear from the antrum of the stomach in the third trimester of pregnancy, but this process fails to occur in approximately 20% of the population [81]. Milk entry into the infant's stomach sharply raises gastric pH followed by slower return to lower pH compared to older children [83]. The outflow of pepsin is also diminished in the newborn infant and increases until 3 months of age [80]. Thus, gastric proteolysis is limited in term and especially in preterm infants because of low pepsin levels combined with low gastric acidity. However, gastric proteolysis does not seem to be critical for further digestion and absorption of proteins.

In the duodenum, several proteases act together to digest proteins into amino acids and oligopeptides (di- or tri-peptides). Pancreatic enzymes are secreted as inactive pro-enzymes that are activated by hydrolysis of a peptide bond. Bile salts in the duodenum induce enterokinase release from the microvillus membrane of the absorptive cells. Entrokinase converts trypsinogen to trypsin that in turn activates all the other pancreatic proteases and the release of more trypsinogen. The concentrations of the proteolytic enzymes (e.g., trypsin, chemotrypsin) excreted from the exocrine pancreas only modestly increase during the first hours to months after delivery, and they are fairly efficient in proteolysis from birth on. This is true for premature infants as early as 23 weeks gestation [84]. However, feeding preterm infants with a high-protein diet stimulates increased trypsin secretion into the duodenum. Lower chymotrypsin concentrations are found in infants who are small for gestational age (SGA) [84] suggesting a deleterious effect of intrauterine growth restriction on pancreatic exocrine function, which may limit postnatal catch-up growth. Concerns regarding protease inhibitors in human milk and colostrum that may interfere with the digestion of proteins in the intestine of breastfed babies [85] were disputed by others [86].

Following the digestion by pancreatic proteases, amino acids and oligopeptides are passively absorbed at the brush

border membrane by secondary active transport via sodium-dependent amino acid co-transporters [87]. The low intracellular sodium concentration creates a gradient for sodium entry that drives the absorption of the coupled amino acid. The energy is indirectly provided by the sodium-potassium ATPase pump. There are different classes of amino acids transporters as well as transporters for oligopeptides. There are also peptidases on the brush border membrane, as well as in the cytoplasm of the absorptive cell. In the embryonic period (4 to 8 weeks) the activity of proteases is mainly detected on the luminal surface of the fetal intestine. Later on, villi are formed from the duodenum up to the ileum. After 9 weeks of gestation differentiation of Lieberkuhn crypts can be observed. The activity of proteases is high in the differentiating microvillous zone of the primitive enterocytes [88]. Thus, the peptide transport system, as well as the brush border and cytosolic peptidases are well developed and functional in preterm infants. In addition, preterm and term newborns have increased small intestinal permeability that allows them to absorb intact macromolecules by active pinocytosis [89]. Amniotic fluid proteins are swallowed in utero and food and other proteins are ingested during the neonatal period. These include albumin, beta-lactoglobulin, immunoglobulins (A and G) and other immunologic factors, hormones (chorionic gonadotropin and growth hormone) and even intact lactoferrin of maternal origin.

42.4.3 Carbohydrate Digestion and Absorption

Carbohydrates provide about 40% of the caloric intake of the newborn. Lactose is the major source of carbohydrates in human milk. Lactase activity appears in the differentiating brush border of the fetal small intestine along with other disaccharidases (mainly sucrase) as early as 12 weeks of gestation [88], although in low levels. Lactase activity exhibits relatively higher levels at 37 weeks of gestation [90]. Lactase activity is still decreased during the first weeks of life, and this is reflected by the limited capacity of term and especially preterm infants to digest and absorb lactose in the small intestine [91]. Nevertheless, preterm infants fed lactose-containing milk or formulas grow well and have no diarrhea. The elevation of breath hydrogen in these infants apparently represents a successful adaptation of the colonic microflora that converts malabsorbed lactose to volatile organic acids that are subsequently absorbed [92]. Glucose polymers are polysaccharides that require hydrolysis by amylase. Since pancreatic alpha-amylase levels are low in young infants and increase only later during postnatal life, glucose polymers are either hydrolyzed by salivary alpha-amylase whose activity increases earlier, or by the intestinal brush border enzyme, glucoamylase. Alpha-amylase in breast milk probably also participates in digestion, because it can survive the mild acidity [93] and low pepsin levels in the stomach of newborn babies.

Glucose and other monosaccharide products of hydrolysis are then absorbed from the jejunum and illeum either by active sodium-dependent D-glucose co-transport carrier system (SGLT1) or by the GLUT family of facilitative glucose transporters [94]. The sodium-dependent D-glucose co-transport carrier systems demonstrate early differentiation of a functional heterogeneity in glucose transport capacity along the human fetal small intestine [95]. These brush border co-transport systems develop in the jejunum and ileum of the fetus as early as 17-20 weeks gestation, and can be differentiated not only by their kinetic properties but also by their differences in both substrate and inhibitor specificities [96]. The facilitative glucose transporter isoforms GLUT2 and GLUT5 are also developmentally modulated with highest levels in adult small intestine. By contrast, GLUT1 expression is higher in the fetal small intestine. In adult small intestine, GLUT5 is localized to the luminal brush-border surface of mature enterocytes. Yet, in the fetal small intestine, GLUT5 is localized along the intercellular junctions of the developing villus. Thus, both the expression and localization of GLUT 5 are developmentally regulated. In mature absorptive epithelial cell GLUT5 is localized to the luminal surface [94]. Postnatal glucose absorption in infants is less efficient than in adults, and the kinetics of glucose absorption is related to gestational and postnatal ages, as well as to the diet and exposure to glucocorticoids [97].

Carbohydrates that are not digested and absorbed in the small intestine reach the colon where they are degraded by colonic bacteria. The last step of this degradation is fermentation that results in the formation of short-chain fatty acids, methane, carbon dioxide and hydrogen. These short-chain fatty acids, particularly butyrate [98], are the preferred energy source for the colonic epithelial cells. In the premature infant, colonic fermentation serves as a major important route for lactose carbon absorption (as discussed above) [92]. Developmental aspects of colonic fermentative activity, effects of systemic antibiotic treatment on colonic micro flora, the effects of various fermentation pathways on energy balance, the capacity for absorption of sugars, short-chain fatty acids, and electrolytes by colonic epithelia, and the effects of fermentation products on metabolism and mucosal cells are all important to premature infants especially in relation to colonic disease or surgical resection [92].

42.4.4 Micronutrient Absorption

The absorption of the different micronutrients matures at varying rates during infancy.

42.4.4.1 Water and Electrolytes

Water is absorbed passively following the gradients of sodium and other electrolytes. Stimulation of intestinal sodium ab-

sorption by adding solutes (sugars or amino acids) that are absorbed by sodium-coupled mechanisms increase net fluid absorption [99].

The intestinal epithelium is more susceptible to diarrhea in young infants. Protracted diarrhea of infancy (PDI) describes infants with loose and frequent stools of sufficient severity to require nutritional support, often in the form of parenteral alimentation [100]. The most common cause for PDI is post-infectious diarrhea with failure of the injured gut to recover rapidly. The causes of PDI can be classified into two categories based on the findings of intestinal biopsy: PDI with normal villi and PDI with villus atrophy. The most common cause of protracted diarrhea with villous atrophy is microvillus inclusion disease, an autosomal recessive disorder that is present upon birth with striking secretory diarrhea. Diagnosis is based on typical electron microscopy findings including the diagnostic finding of microvilli inside involutions of the apical membrane [101]. During pregnancy, ultrasounds demonstrate multiple fluid-filled dilated intestinal loops and polyhydramnios. Other causes of congenital diarrhea with villus destruction include tufting enteropathy, autoimmune enteropathy, and IPEX syndrome [100], as well as carbohydrate-deficient glycoprotein syndrome [102], and enterocyte heparin sulfate deficiency [103].

In the category of normal villous PDI, congenital ion transport defects cause secretory diarrhea presenting at birth. The most common congenital ion transport defect is in the chloride-bicarbonate anion exchanger that is located in the distal ileum and colon. This trans-membrane protein belongs to the sulfate transporter family that have three known members in humans, all associated with a distinct genetic disease [104]. Members of the gene family can transport other anions as well (chloride, sulfate, and oxalate) [105]. In these cases of congenital chloride diarrhea, HCO_3^- is not secreted into the gastrointestinal tract lumen, leading to alkalosis. Fecal chloride concentration exceeds the sum of fecal sodium and potassium levels, establishing the diagnosis [104]. Congenital sodium diarrhea, a disorder of the intestinal H^+/Na^+-exchanger located in the small intestine and colon, is characterized by hyponatremia, alkaline diarrhea, and a high concentration of stool sodium [106]. In the other congenital ion transport defects (sodium co-transporters), abnormal reabsorption of sodium is involved, which usually results in hyponatremia [100].

The colon has an important role in absorbing sodium and water from the intestinal lumen. Active absorption of sodium ions can be increased 3-4 fold by the presence of aldosterone [107], suggesting that colonic absorption of sodium is central to maintaining salt and water homeostasis. Colonic mucosa in the fetus has well-developed villi that allows it to be involved in nutrient absorption in contrast to the adult [108]. Thus, in addition to its role in salt and water homeostasis, it can also absorb essential nutrients by active (sodium-coupled) transport of glucose and amino acids that may also compensate for decreased absorption in the developing small intestine.

42.4.4.2 Minerals and Trace Elements

Trace elements and mineral absorption depends on the milieu in which they are presented to the intestine. The bioavailability of iron, calcium and other minerals for absorption is better from human breast milk [109]. Preterm infants may absorb as much as 50% of the iron in breast milk, as opposed to formulas. This is true for other minerals that are more bioavailable for preterm infants in human milk. This does not alter the need to supplement some of these nutrients (mainly calcium and phosphorus) to preterm infants to compensate for their increased demands [110].

Calcium and phosphorus absorption and requirements are further discussed in Chapter 49.

42.4.4.3 Vitamins

Human milk is a good source for most vitamins. In healthy breastfed infants of well-nourished mothers, there is little risk of vitamin deficiencies and the need for vitamin supplementation is rare. The exceptions to this, to avoid deficiencies, is a need for vitamin K supplementation in the immediate newborn period and vitamin D in breastfed infants with dark skin or inadequate sunlight exposure [111, 112]. Most vitamins appear to be absorbed adequately in term and preterm infants.

Fat Soluble Vitamins

Fat soluble vitamins include vitamins A, D, E and K. Vitamins A (retinoids) and E (tocopherols) are two potent antioxidant nutrients that also play a significant role in immune function.

Vitamin D (calciferol) plays a major role in intestinal calcium absorption and bone mineralization [112]. Cholesterol derived precursor uses UV sunlight to convert to previtamin D_3, which is then converted to an inactive form of vitamin D_3. Vitamin D_3 is then hydroxylated in the liver (25-(OH)D) and in the kidneys to make active vitamin D (1,25-$(OH)_2$D). Vitamin D crosses the placenta mainly in the form of 25-(OH)D. The transport is passive or facilitated, with lower values in the fetus, and therefore fetal 25-(OH)D concentrations correlate with those of the mother. Exogenous vitamin D_3 (from animal source), or D_2 (ergocalciferol, synthesized by plants) can also be absorbed in the duodenum and the jejunum, although vitamin D_2 appears to be absorbed to a much lesser extent than vitamin D_3 [113].

Vitamin K intervenes in the synthesis of coagulation factors particularly in prothrombin synthesis. Fat-soluble vitamins are totally dependent on micelles in order to be presented to the brush border membrane. Absorption is also dependent on the presence of bile salts in the small intestine [114]. Ingested carotene and dietary retinyl esters are converted to free retinol in the proximal small intestine by pancreatic hydrolases and are the dietary sources for vitamin A. Retinol absorption

into the intestinal cells is facilitated by retinol binding protein, RBP II, which is found almost exclusively in absorptive cells. In the enterocyte, fat soluble vitamins are re-esterified, and incorporated into the developing chylomicron. Chylomicrons travel through the lymphatic system and then enter the bloodstream. Some of the vitamins use protein carriers for transport in the bloodstream (retinol binding protein, RBP I for carrying retinol to target tissue and D-binding protein to take hydroxylated vitamin D from the liver to the kidney for further hydroxylation). Excess is stored in liver and adipose tissue, and released as needed by the body. Risk of toxicity may be relevant for high doses of vitamin D, A and E and is less relevant for vitamin K. Disorders affecting fat absorption, such as short bowel syndrome, cholestasis and other diseases associated with bile acid deficiency may cause fat-soluble vitamin deficiency. Vitamin K2 (menaquinones) is synthesized by bacteria in the large intestine. Because the transfer of vitamin K across the placenta during pregnancy is poor and the sterile gut of the infant is unable to produce menaquinones, newborn babies are routinely given supplements of vitamin K [114].

Water Soluble Vitamins

Water-soluble vitamins are required as enzyme cofactors in a wide variety of metabolic reactions. Riboflavin (vitamin B2), niacin, and vitamin C (ascorbic acid) are essential for oxidation-reduction reactions. Thiamine (vitamin B1) and biotin are involved in macronutrient metabolism. Folate, vitamin B12, pyridoxine (vitamin B6), and riboflavin play important roles in the regulation of S-adenosylmethionine production and DNA synthesis. Each of these water-soluble vitamins requires its own membrane transport process for absorption across the enterocyte. Most of the water-soluble vitamins are absorbed from the proximal small intestine [114, 115]. Folate, biotin, and riboflavin can be transported across colonic epithelial cells as well, although the clinical significance of this is uncertain [115]. Riboflavin is light sensitive and is rapidly photodegraded. Phototherapy is a possible cause for riboflavin deficiency in neonates [116]. This may affect antioxidant mechanisms in preterm infants. The absorption of vitamin B12 (cobalamin) is complex and unique in requiring multiple steps from the stomach to the ileum and the involvement of at least four different binding proteins [115]. In the gastric acidic environment it is released from the food to form complexes with R-binders that are proteins found in the saliva, gastric juice, bile and milk and have high affinity for vitamin B12. At the alkaline environment of the duodenum pancreatic proteases degrade R-binders allowing vitamin B12 to bind to the intrinsic factor that is produced by the parietal cells of the stomach. The complex of vitamin B12 and intrinsic factor is absorbed intact in the distal ileum. Inside the ileal mucosal cells, vitamin B12 is released from the intrinsic factor and binds to a specific transport protein, transcobalamine II, to be delivered to the liver via the circulation. It is excreted mainly in the bile, but has a very long half-life due to very effective enterohepatic circulation. It is actively transported across the placenta so that newborns have concentrations twice as high as mothers [114]. Hepatic stores correlate with the duration of pregnancy and are thus large in full-term newborns, but lower in preterm infants. Clinical manifestations of vitamin B12 deficiency appear only in late infancy, because of the hepatic stores and the long half-life [114]. There are several congenital defects that can affect the complex absorption of vitamin B12, but they are rare compared to dietary deficiency that is not evident in the neonatal period.

42.5 Host Defense

Colonization of the gastrointestinal tract of newborn infants starts immediately after birth and occurs within a few days. Initially, the type of delivery (passage through the birth canal versus cesarean section) and the type of diet (breast milk versus formula feeding) affect the colonization pattern [117]. Other environmental factors such as developed versus developing countries and use of antibiotics in the neonatal period may also affect the microflora composition [118]. The immune system of preterm infants is immature. Thus, preterm infants are prone to a delayed gastrointestinal colonization, reduced microbial diversity in the bowel, acquisition of antibiotic-resistant strains, loss of strains associated with antibiotic treatment, and increased intestinal bacterial translocation [119]. The gut of extremely low birthweight infants is colonized by a paucity of bacterial species [120]. This can favor the overgrowth of microorganisms such as *Enterobacteriaceae* and *coagulase-negative staphylococci*, which are the most frequent pathogens of nosocomial infection in neonatal intensive care units [121]. These factors also make the preterm infant more susceptible to antibiotic-resistant infections, systemic inflammatory response syndrome and necrotizing enterocolitis (NEC) [122]. Breast milk and reduction of antibiotic exposure have been found to be important in increasing fecal microbial diversity in these premature infants [120]. Microbes of the intestinal tract serve several roles, including nutrition, growth, and maintenance of function and development of the immune system [123].

The gut is in continuity with the environment and thus exposed to bacteria and antigens. The gastrointestinal tract presents the largest surface area in the body exposed to microbes and other antigens. On the other hand, it needs to allow the entrance of nutrients and other beneficial molecules. Host defenses include non-specific and specific components. The innate immune system responds in a non-specific way, and the adaptive immune system responds specifically by humoral and cellular components that specifically react to particular antigens.

Defense factors in human milk include antimicrobial agents (secretory IgA, lactoferrin, lysozyme, glycoconjugates,

oligosaccharides, and digestive products of milk lipids), anti-inflammatory factors (antioxidants, epithelial growth factors, cellular protective agents, and enzymes that degrade mediators of inflammation), immunomodulators (nucleotides, cytokines, and anti-idiotypic antibodies), and leukocytes (neutrophils, macrophages, and lymphocytes). The presence and function of immunomodulatory and anti-inflammatory factors present in human milk help protec the mature newborn as well as the premature infant against infections. Some of these factors present in human milk may actively modulate the synthesis and maturation of the recipient immune system. This complex interactive system of bioactive substances in human milk seems to be designed to operate in a complementary manner with other non-inflammatory mechanisms in order to resist digestion of these factors in the recipient gastrointestinal tract, and to supplement the infant with developmentally delayed immune factors. This is consistent with the theory of the evolution of the mammary gland from the immune system [124]. In addition to the well-known passive protection against infections during lactation (mainly via secretory IgA antibodies, and also via several other factors like bactericidal lactoferrin), breastfeeding may also actively stimulate the immune system of the infant (via anti-idiotypic antibodies, cytokines, growth factors, T and B lymphocytes and macrophages) with other long-term positive effects (e.g. better immunological response to infections and vaccines) [125, 126].

Gastric acidity and pancreato-biliary secretions also decrease the load of viable microorganisms and intact dietary protein antigens. These defenses are all decreased in the newborn, especially the preterm infant, as described above. The use of H2 blocking agents or proton pump inhibitors that further decrease gastric acidity contribute to a decreased defense and may result in higher rates of infections [127].

Mechanical factors that appear early in gestation are also involved in the a host defense by creating a physical barrier of mucous secretion and by gastric emptying and intestinal peristalsis that prevent stasis and bacterial overgrowth. In preterm infants gastric emptying, MMCs and peristalsis may be delayed, as discussed above. Intestinal mucus secreted into the lumen from storage vacuoles in the goblet cells, found throughout the small and large intestine, plays a significant role in intestinal defense. It contains mucins, glycoproteins, immunoglobulins, glycolipids and albumin that form a slippery gel over the intestinal surface that enhances forward propulsion. Mucous gel can also trap large foreign antigenic molecules thus preventing their diffusion into the gut wall. Mucin secretion changes in amount and composition during development and maturation of the newborn infant and in response to stress and hypoxia [128]. Microvilli also constitute a significant barrier because of their size and negative charge that prevent large macromolecules from penetrating.

The intestinal single-cell layer of columnar epithelial cells is present by the end of the first trimester. It is the cellular site of innate immunity of the intestine separating the host from the intestinal lumen. It is arranged in crypts and villi. Stem cells at the base of the crypts proliferate and differentiate into enterocytes that migrate to the villus tip. They eventually slough into the lumen by physiologic apoptosis (anoikis), thus allowing reconstitution of the epithelium every 5 days [129]. This renewal mechanism serves as a defense mechanism against epithelial injury. Epithelial integrity is further protected by intracellular contacts of membrane proteins and cytoskeletal anchor proteins that form the apical junction complexes (AJCs) that are the basis for the series of tight junctions between the cells. The AJCs and their cytoskeletal connections are dynamic structures that closely regulate the flow of fluids, electrolytes ions and even small molecules between the cells but prevent passage of larger molecules [130]. Epithelial cells also regulate transcellular permeability to ions and small molecules through alterations in the expression of selective membrane ion channels and pores. Control of water and chloride secretion through the channels can result in secretory diarrhea that can flush away toxins and pathogens from the lumen [131].

In addition to mechanical barriers and mucin secretion, the gut elaborates chemical defenses secreted by the absorptive enterocytes and Paneth cells. Paneth cells are specialized secretory enterocytes located at the base of the small intestinal crypts, where they can control microbial populations and protect neighboring stem cells. Paneth cells secrete lysozyme, phospholipase A2, and antimicrobial peptides. Antimicrobial peptides are divided into defensins (alpha and beta), which are the predominant class, and cathelicidins. These small cationic peptides are able to insert into the membranes of a broad range of microbes (including bacteria, fungi and enveloped viruses), where they play an active role in oxygen-independent killing. It is possible that low levels of Paneth cell defensins, characteristic of normal intestinal development, may predispose preterm infants to necrotizing enterocolitis (NEC) [132].

An important nonconstitutive defense component of the gut innate immunity is the carefully programmed and regulated inflammatory response that is activated when potentially injurious stimuli cross the barrier of the intestinal epithelium. This activated inflammatory response induces recruitment of leukocytes (polymorphnuclear and macrophages) as well as complement, defensins and cytokines to aid in defense. Multiple endogenous or exogenous signals can start the inflammatory process by inducing the local release of soluble inflammatory mediators and chemotactic agents that increase vascular permeability and attract inflammatory cells, resulting in edema and inflammation [133].

The human genome contains evolutionary ancient components of innate immunity that are transmitted in the germ cells without exposure to the microbes, and encode at least 10 toll-like receptors (TLRs). TLRs are trans-membrane receptors that recognize pathogen-associated molecular patterns (PAMPs) or microbial associated molecule pattern (MAMPs) that are found only on microbes, like lipopolysaccharides, peptidoglycans and lipoproteins. The binding of MAMPs to TLRs or intracytoplasmic NOD proteins results in the activation of cytoplasmic signaling circuits. These pathways include the

classic nuclear factor kappa-B (NF-kappa-B), the mitogen-activated protein kinase (MAPK), and the interferon regulatory factor (IRF) pathways. During the initial events of bacterial perception by the intestinal epithelial cells all these three pathways are activated within minutes, leading to nuclear translocation of transcription factors and transcriptional activation of a battery of effector molecules [134]. This results in induction of the expression of enzymes important for bacterial killing and wound healing, as well as antibacterial peptides, cytokines, adhesion molecules, chemotactic messengers and antiapoptotic proteins [135]. The sterile fetal gut is naïve to MAMPs in utero. Within the first days the neonatal gut is challenged by multiple MAMPs as the luminal flora is introduced. TLRs are expressed at the basolateral aspects of fetal intestinal crypts as early as 20 weeks of gestation [136].

Membranous or microfold cells, commonly referred to as M cells, are specialized epithelial cells of the gut-associated lymphoid tissues (GALT) that play a major role in the intestinal immune system by delivering luminal antigens to the underlying immune cells. M cells in the intestine lack well-developed microvilli and allow macromolecular transport, as opposed to the absorptive enterocytes with their developed villi. M cells are present only in follicles overlying lymphoid tissue to which they can immediately present foreign antigens and microorganisms that they transport as macromolecules [137].

Subepithelial cells include follicular dentritic cells that are located in the lymphoid follicles and are important in nonphagocytic presentation of antigens to T-cells as well as B-cells. Peyer's patches are aggregates of lymphoid tissue that are present by 19 weeks of gestation, but become more prominent in the jejunum and ileum between 24-40 weeks. M cells occur in follicle-associated epithelium over areas of Peyer's patches [137]. Mast cells bind immunoglobulin E and subsequently release histamine and serotonin that stimulate mucus production by adjacent goblet cells, increase enterocyte permeability, chemotaxis of granulocytes, contraction of smooth muscle cells and lymphocyte function.

The immune system of host defense is composed of cellular and humoral components. T and B lymphocytes are produced as early as 12 weeks gestation, and are present with macrophages in the fetal intestine by 20 weeks gestation. Interspersed between the intestinal epithelial enterocytes are blood-derived intraepithelial lymphocytes (IELs) that are immune components of the host defense. They are located in the basolateral side of the epithelial layer where cells are exposed to various food and microbial antigens. They are cytotoxic and capable of producing cytokines in order to protect the host from invasion of microorganisms through the gut. Immature human enterocytes react with excessive pro-inflammatory cytokine production after inflammatory stimulation in premature infants exposed to initial colonizing bacteria and this may lead to necrotizing enterocolitis (NEC) [138]. On the other hand antigenic stimulation of the lymphoid tissues cannot be demonstrated before 46 weeks of postmenstrual age, which poses a challenge for preterm infants whose gut can absorb macromolecules directly by pinocytosis. Secretory IgA is present by 22 weeks of gestation, although in small amounts, because the newborn intestine has few IgA-producing plasma cells. In addition preterm infants are not able to form antibodies in response to exogenous food proteins.

Enteral nutrients and food supplements may also contribute to host defense. These include amino acids (glutamine and arginine), nucleotides, long-chain fatty acids, probiotics and prebiotics.

Glutamine contributes to maintenance of small intestinal inter-epithelial junctional integrity [139], intestinal epithelial cells growth and proliferation [140], inflammatory responses and active healing. Glutamine deficient diet increased bacterial translocation *in vitro* by altering intestinal tight junctions [139, 141], and glutamine supplementation decreased the incidence of sepsis in very-low-birth-weight infants [142]. Glutamine and glutamic acid together with taurine are the only free amino acids whose concentrations are increased in human milk during the first 3 months of lactation [143].

Arginine also plays an important role in immune function. Low plasma arginine levels in preterm infants were associated with increased risk for subsequent NEC [144], and its supplementation may prevent NEC [145].

Exogenous nucleotides influence cell proliferation, differentiation and apoptosis in the crypt and villus epithelium of fetal and neonatal small intestine [146]. Nucleotides in infant formulas also enhance humoral antibody responses to immunizations [147], and have a favorable effect on the lipid profile of preterm infants [148].

Long-chain polyunsaturated fatty acids (LCPUFA), Arachidonic Acid (AA) and Docosahexaenoic Acid (DHA), which are metabolites of the essential n-3 and n-6 fatty acids are known to modulate inflammation. Supplementation of preterm infants' food with LC-PUFA may reduce the risk of NEC [149, 150] and also has beneficial effects on the developing visual system and cognitive development during the first year of life [151].

Probiotic bacteria have been shown to reinforce the different lines of gut defense, which are immune exclusion, immune elimination, and immune regulation. They also stimulate nonspecific host resistance to microbial pathogens, thereby aiding in pathogen eradication. Documented effects include the alleviation of intestinal inflammation, normalization of gut mucosal dysfunction, and down-regulation of hypersensitivity reactions. Thus, modification of gut microflora by probiotic therapy may be suitable for clinical conditions associated with gut-barrier dysfunction and inflammatory response [152]. Consequently, probiotics are used in the treatment of acute diarrhea in children [153]. Probiotics modify the fecal flora, can reduce the overgrowth of pathogens in the bowel of preterm infants, and thus contribute to the reduction of the incidence of nosocomial infections in neonatal intensive care units [154]. *Bifidobacterium* species are the most common bacteria in human milk fed infants. Infants consuming

Bifidobacterium breve supplemented feedings had higher rates of fecal bifidobacteria colonization with some clinical advantages including decreased gastric aspirates and improved weight gain and feeding tolerance with no identified side effects [155]. Significant reduction in the incidence of NEC and NEC-associated fatalities was shown by Hoyos et al in preterm infants given daily doses of live *L. acidophilus* and *B. infantis* with no documented complications [156]. Dani et al found that the incidence of NEC (Bell stage ≥ 2) was lower in preterm infants supplemented with *Lactobacillus* GG (LGG) [154]. Lin et al have shown that supplementation of human milk [157] or formula feedings [158], with a mixture of *Lactobacillus acidophilus* and *Bifidobacterium infantis* [157] or *bifidum* [158], significantly reduced the combined incidence of NEC or death in very-low-birth-weight (VLBW) infants. There was a significant effect on NEC (Bell stage ≥ 2) itself as well. Bin-Nun et al showed that a probiotic mixture (*Bifidobacterium infantis*, *Streptococcus thermophilus*, and *Bifidobacterium bifidus*) had a significant protective effect on NEC of any Bell stage in VLBW infants, even when Bell stage ≥ 2 NEC was only considered [159]. Recent meta-analysis suggests that probiotics could significantly reduce the risk of NEC in VLBW preterm infants born after less than 34 weeks gestation [160], yet current available data are not sufficient to define the most suitable probiotic product or to settle the issue of the safety [161] of routine use of probiotics in young premature infants with immature defense systems [162].

Another approach is to use prebiotic supplementation. Prebiotics are defined as non-digestible substances that, when ingested, selectively promote the growth and establishment of beneficial probiotic-like bacteria normally present in the gut [163]. There are few studies on the use of oligosaccharide prebiotic products in preterm infants that have been shown to increase the counts of fecal bifidobacteria, reduce stool pH, reduce stool viscosity, accelerate gastrointestinal transport and improve mineral absorption [164–167] with no negative impact on weight gain. More randomized trials in preterm infants are needed to support their potential beneficial effects on feeding tolerance and on the reduction of the incidence of NEC and hospital acquired infections [166, 168]. Currently there is not enough data available to recommend the general use of prebiotics or probiotics in preterm infants [162, 163].

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43

Hormones and Gastrointestinal Function

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43.1 Introduction

Development is a continuous process. Nutrition, environment and stress modulate development through gene expression in an epigenetic manner. Prenatal and perinatal nutrition can be imprinting factors and turn on different genes that provide different phenotypes, such as the thrifty phenotype [1]. Indeed, the nutritional support of gastrointestinal growth and function is an important tool in the clinical care of newborn babies, in particular preterm neonates. Before birth, although amniotic fluid is not the main source of nutrition for the fetus, it contributes up to 15% of fetal nutritional requirements and plays a key role in its development and maturation [2, 3]. Accordingly, by 20 weeks of gestation, the anatomy of the fetal gut resembles that of the term neonate. However, the process of intestinal absorption is only partially mature before 26 weeks of gestation: gastro-entero-pancreatic peptides are secreted at a basal rate and can be completely stimulated or inhibited after delivery, in particular through contact with nutrients [4, 5]. At the age of 2 years, the intestine is fully functional [6]. Gut hormones, peptides, and growth factors clearly have a role in gut growth after birth and directly and indirectly mediate the trophic actions of enteral nutrition in a manner that is still incompletely understood [5]. By contrast, hormones and growth factors, which are present in breast milk, also seem to exert trophic activities on gut development and immune function. The interplay is complex [1, 3]. Little is known about the development of these regulatory systems in the human neonate and, as a consequence, premature infants experience significant morbidity and mortality associated with feeding problems [6]. Present clinical nutritional support for preterm babies consists of enteral and parenteral nutrition but both have associated complications [6, 7]. Since enteral feeding is important for gut development, acute or chronic gastrointestinal diseases could be caused by feeding with formula rather than human breast milk. Formula milks contain higher amounts of proteins and lack many endogenous hormones and growth factors [5, 6, 8]. A better understanding of factors linked to gastrointestinal function and energy metabolism could result in improved strategies for supporting nutrition of preterm newborns as well as their later development.

The aim of this chapter is to review the state of knowledge about gut peptide secretion during the neonatal period. It has to be emphasized that much information remains anecdotal and conflicting, probably due to small sample sizes, various methods of measurements or distribution of subjects.

43.2 Etiology, Pathogenesis and Clinical Aspects: Gut Peptides

In recent years, many peptides produced by the gut have been discovered that are closely involved in the development of gut function as well in the acute and chronic regulation of weight and energy balance. They are also affected by nutrient ingestion and could influence pancreatic secretion and beta-cell proliferation and survival. There is considerable data about the relationship between gut peptides and obesity and the development of type 2 diabetes during adulthood. However, investigations of their role during the neonatal period are ongoing.

43.2.1 Peptides from Proglucagon

43.2.1.1 Glucagon-like Peptide 1 (GLP-1)

GLP-1 is a peptide of 30 amino acids expressed and secreted by endocrine L cells of intestinal mucosa in the ileum and colon. GLP-1 derives from the same precursor as glucagon. Proglucagon is also expressed in the brain, in particular in the arcuate and paraventicular nuclei. Three hormonal products,

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GLP-1, GLP-2 and oxyntomodulin (OXM) are derived simultaneously from the processing of proglucagon by neurons. In the gut, truncated peptides of proglucagon (GLP-1 7-36amide or GLP1 7-37) are the active forms, while the full-length native GLP1 is inactive [9]. Almost all secreted GLP-1 from L cells is amidated resulting in improved stability. Only 10-15% of the secreted GLP-1 is intact when it reaches the systemic circulation. This rapid initial whole body clearance is due to metabolism in the liver and to the activity of DPP-IV, which is expressed by enterocytes and by endothelial cells of capillaries of the lamina propria [9].

GLP-1 has several actions, including increasing insulin and suppressing glucagon postprandial secretion, thus modulating the pancreatic glucose-response [10, 11]. The amplification of insulin secretion in response to glucose levels by gut hormones has been termed the incretin effect [9].

GLP-1 levels have been shown to be modulated by ingested macronutrients through direct activation of secretion by L cells, probably by an interaction with microvilli [9]. The meal size and composition seem to be key factors for GLP-1 secretion in adulthood, where there is a correlation with gastric emptying. Small meals, particularly when nutrients were rapidly absorbed, did not result in a measurable secretion of GLP-1 but did stimulate large amounts of glucose-dependent insulinotropic polypeptide (GIP). By contrast, large meals containing more complex nutrients particularly lipids, were rapidly followed by high GLP-1 secretion [9, 12].

GLP-1 plays a role in mediating appetite and feeding behavior. A reduction in hunger coupled with the sensation of fullness, has been reported after its administration [13]. Consistent with animal studies, in humans GLP-1 induces a mild but significant dose-dependent inhibition of food intake in both lean and obese subjects as well as in patients with type 2 diabetes [14]. A role for GLP-1 in the development of gut function was hypothesized because of its incretin action and its regulation by nutrients.

There are limited studies of GLP-1 in relation to the developing fetus and gut maturation from prenatal to postnatal life. GLP-1 has been implicated in the regulation of pancreatic beta-cell mass during the neonatal period [9, 15]. Preterm and term neonates also have higher fasting and post-feeding GLP-1 levels, probably because of immaturity of dipeptidyl peptidase-IV (DPP-IV, which degrades GLP-1) or a reduced glomerular filtration rate. Increased activity of GLP-1 could also regulate entero-endocrine systems by increasing beta-cell mass and regeneration. However, it is not known whether increased GLP-1 levels are also derived from a higher gut or pancreatic production rate [16]. GLP-1 levels during feeds have recently been shown to be higher in preterm neonates than in older children or adults without being linked to hypoglycemic episodes. Most infants of this study were fed by nasogastric tube, suggesting direct stimulation of enteric pathways without activation of cephalic vagal tone [7]. It has been suggested that GLP-1 has a key ontogenic role in the regulation of the immature gut [7]. There are few data for other populations, in particular small for gestation age (SGA) subjects who are at a higher risk of developing glucose intolerance or diabetes mellitus, although a preliminary study involving adults born SGA described fasting and postprandial GLP-1 levels similar to healthy controls [17]. No studies have measured GLP-1 secretion in SGA or AGA subjects at birth or during the first two years of life.

43.2.1.2 Glucagon-like Peptide 2 (GLP-2)

GLP-2 is a 33 amino acid peptide also produced by the endocrine L cells by post-translational processing of proglucagon and secreted with GLP-1 in response to food ingestion [9]. It is also produced in the brain [18]. It is rapidly cleaved by DPP-IV with a half-life of 7 minutes, which is longer than that of GLP-1 [7]. It binds and activates its own receptor, the GLP-2 receptor, which is homologous to those of GLP-1, GIP, and glucagon [5, 7]. Like GLP-1, it is also modulated by ingested macronutrients, namely carbohydrates and lipids [19] and short chain fatty acids [5]. In adults, after an oral feed, GLP-2 secretion shows a biphasic increase with a first peak within 15 minutes and a second one after 1 hour, suggesting not only a direct stimulation by nutrients but also the involvement of other factors, in particular GIP and vagus nerve [5, 18].

In contrast to GLP-1, the role of GLP-2 in the regulation of feeding and during fasting is unclear. There is no evidence that the systemic injection of GLP-2 affects food intake in animals. However, the intracerebroventricular administration of the peptide suppresses food intake in rodents in a similar fashion to GLP-1 [20]. In humans, no variations in feeding behavior or gastric emptying have been observed after its administration [21].

Unlike GLP-1, GLP-2 is mainly a trophic peptide of intestinal mucosa and seems to slow gastric motility during fasting [5, 7]. Its administration inhibits gastric secretion [5]. GLP-2 increases gut epithelial absorptive capacity and mucosal growth by acting both on endocrine pathways and enteric neurons [7, 18, 22]. Several studies have suggested that GLP-2 is involved in controlling the absorptive capacity of the proximal bowel. Thus, following gut resection in both animals and humans, the residual bowel secretes higher amounts of GLP-2 after feeding , suggesting a process of adaptation [22]. Dietary carbohydrates stimulate the production of short chain fatty acids and it has been suggested that their malabsorption may trigger increased GLP-2 secretion [5, 23]. GLP-2 has been shown to also act on gut barrier functions by reducing bowel permeability to macromolecules, decreasing bacterial translocation and suppressing the local expression of proinflammatory cytokines [5, 15, 18, 22].

A trophic role for GLP-2 in the development and maturation of gastrointestinal function has been investigated, although its specific functions remain largely unknown. It is active in the immature bowel, with a peak of secretion during the late weaning period [5, 18]. However, studies of mutant mice have demonstrated that GLP-2 is not necessary for

bowel development during fetal life [24], despite its secretion mechanism being established by 24 weeks of gestation in humans [25]. In addition, GLP-2 levels in utero would be expected to be lower respect than during postnatal life because of higher levels and DPP-IV activity in the placenta [26].

Both fasting and postprandial levels of GLP-2 are higher in neonates when compared to older children or adults. As with GLP-1, these findings could be due to either an increase in production and/or a decrease in metabolism by DPP-IV immaturity or lower glomerular filtration [7, 25]. Increased GLP-2 levels in preterm babies may stimulate gut development. It is notable that normal infants aged 48 weeks have lower postprandial GLP-2 levels compared with delivery at 40 weeks. These data suggest that gestational age may influence GLP-2 secretion and that its peak of secretion could correspond to the time of the most rapid growth of gut, which ranges between 30 and 40 weeks of gestational age [7]. However, unlike animals and adults, infants with intestinal dysfunction or residual small bowel with intact colon do not appear able to produce GLP-2 in response to stimulation by feeding [27]. The reason of these discordant findings remains unexplained. The lack of GLP-2 secretion could be due to resection of the L cell bearing ileum as well as an age-related inability to produce an appropriate response or to ischemia that may alter GLP-2 secretion in the remnant gut [27]. These observations suggest that stimulation of GLP-2 secretion or GLP-2 supplementation may have therapeutic roles for infant gut dysfunction or for preterm newborns and further studies to address the ontogeny of the GLP-2 axis are needed.

43.2.1.3 Oxyntomodulin (OXM)

OXM is a 37 amino acid peptide derived from post-translational processing of the tissue-specific proglucagon molecule in intestinal cells, in particular in L cells in the distal portion of small bowel [16, 28]. OXM co-localizes with GLP-1 and Peptide YY (PYY) expression and secretion [16, 28]. Limited data are available about a possible feedback system among these peptides in adults as well as in neonates. OXM is also produced in the brain [29]. Like GLP-1 and GLP-2, it is inactivated in large amounts by DPP-IV [30]. The only known receptor for OXM is the same as for GLP-1, but the existence of other unknown receptor subtypes has been suggested [16, 30].

Similarly to GLP-1, OXM is released into the circulation after food ingestion in proportion to the caloric intake [29]. It has a lower incretin effect than GLP-1 [16, 30]. OXM also acts as an anorectic peptide [16, 28, 30]. Its administration induces a prompt decrease in the sensation of hunger, food intake and gastric emptying [31, 32]. However, the mechanisms of action for OXM have not been fully investigated. The reduction in energy intake after OXM administration is followed by weight reduction in both animals and humans [33]. This effect is not only due to the OXM anorectic action but also to an increase in energy expenditure [30]. OXM levels also increase after gastric bypass, in a similar fashion to GLP-1 [34]. No studies have compared activities of OXM with those of GLP-2.

Although the role of OXM is intriguing, no studies have been performed during the neonatal period or childhood. This proglucagon-derived peptide, which shares both effects of GLP-1 and GLP-2, could be a good marker of nutritional status and gut development in infants [35].

43.2.2 Glucose-Dependent Insulinotropic Polypeptide (GIP)

GIP is a peptide of 42 amino acids, which is synthesized by K cells located mainly in the proximal small intestine (duodenum and jejunum) [9]. GIP is frequently found to be colocalized with GLP-1, but rarely with PYY. As with other incretin hormones, GIP is cleaved and inactivated by DPP-IV with a half life of approximately of 5 minutes [36, 37]. GIP receptors are expressed in several tissues, including alpha- and beta-cells, stomach, adipose tissue, pituitary, heart, and brain [36, 37].

GIP secretion rises 15 minutes after the ingestion mainly of glucose and fats [36] and is also inhibited by glucagon [36, 38]. GIP can be considered as another incretin hormone [9]. However, it is clear that GIP alone cannot account for the full incretin effect normally associated with a mixed meal, with the main effect exerted by GLP-1 [9, 39]. GIP, like GLP-1 is also able to promote beta-cell neogenesis, proliferation and differentiation [36].

The administration of GIP improves glucose tolerance and insulin sensitivity in animals [40, 41]. However, GIP receptor knockout mice subjected to a high fat diet do not gain weight and have a preserved insulin sensitivity [42]. The marked weight decrease after gastric bypass seems to depend on surgical ablation of duodenal GIP secretion [43], suggesting it acts rather as an obesiogenic agent than having an anti-obesity or incretin hormone role [44–46].

There are few data relating to the major incretin hormones, in particular GLP-1. Studies on GIP in children investigated the integrity of its response during early life. In both term and preterm neonates, the enteroinsular axis has a functional role, responding to glucose with an increased plasma GIP concentration, which partially and progressively contributes to an enhanced insulin response to enterally infused glucose as well as to oral feeding [17]. One preliminary study has shown that GIP levels are lower in both term and preterm neonates in cord blood at birth than in adults. Moreover, its bloodstream concentration increases 2 hours after birth [47]. Basal GIP levels increased progressively when babies were fed orally and also increased after meals by the 24th day of life [48]. SGA and AGA babies have similar fasting and post-prandial GIP levels [49].

The infants of diabetic mothers and large for gestational age (LGA) newborn babies have a more rapid insulin response

to oral glucose without differences in GIP levels when compared to AGA controls [50]. Moreover, there is a higher GIP/insulin ratio in the amniotic fluid of diabetic pregnant women who delivered overweight infants compared with non-diabetic pregnant women or those who delivered normal weight babies [51]. These data suggest that the role of GIP on fat accrual is precocious and linked to glucose metabolism.

43.2.3 Peptide YY (PYY)3-36

PYY, a member of the NPY family, is a 36 amino acid peptide that is secreted during feeding from the L cells of the small and large bowel. It is released into the circulation in two forms, PYY1-36 and PYY3-36, which is most abundant in the bloodstream [11, 16, 51]. PYY has been defined as an orexigenic peptide. In particular PYY3-36 shows anorectic effects after both peripheral and central administration acting on Y2 receptor at the hypothalamic level [51, 52].

Plasma PYY3-36 levels increase after a meal [52]. In humans the administration of PYY3-36 is followed by a 30% reduction of ingested nutrients and is coupled with fullness sensation [53] and nausea at higher doses [9]. PYY is also involved in regulating glucose homeostasis. Unlike other gut peptides, it directly inhibits glucose-mediated insulin secretion [54]. These effects are associated with an improved insulin action on glucose disposal independent of effects on food intake and weight (55). PYY also directly promotes higher fat combustion in animals [56]. Fasting plasma PYY3-36 levels are lower in obese than in lean subjects [16], suggesting an involvement in the long-term control of weight.

There are increasing data relating to the role of PYY in neonates [57, 58]. In newborn babies, PYY3-36 levels seem to account for almost half the total circulating PYY [59, 60]. It correlates negatively with gestational age and anthropometric measures and is higher in the cord blood of preterm than full-term babies or adults, possibly to compensate for poor growth [59–61]. PYY also increases after enteral feeding with formula milk in preterm babies affected by necrotizing enterocolitis [61, 62]. Its fasting concentration decreases in 9 month old infants [63], suggesting that a postnatal surge is a feature of early adaptation to extrauterine life with possible effects on energy homeostasis and on the gut, including absorptive, trophic and proliferative actions [59, 60, 63]. More data are needed to confirm this hypothesis.

43.2.4 Peptides from Preproghrelin

43.2.4.1 Acylated (AG) and Unacylated (UAG) Ghrelin

Ghrelin is a 28 amino acid peptide, recently isolated from the stomach, but also expressed by other tissues such as pancreas, testes, placenta, pituitary and hypothalamus [64]. Ghrelin has been identified as an endogenous ligand of the orphan GH Secretagogue (GHS)-receptor type 1a [65, 66]. It circulates in blood in two forms, acylated (AG) and unacylated ghrelin (UAG) [64, 65]. The acyl group which binds ghrelin at the serine-3 residue, seems to be essential for the binding to GHSR1a and the resulting neuroendocrine functions, namely GH secretion [64, 65]. UAG, which is devoid of the acyl group, represents the most abundant circulating form [67, 68]. UAG is biologically active, although it does not have direct neuroendocrine actions [64, 65, 69] suggesting the existence of some GHS-R subtypes [64, 68, 70]. The mechanism of acylation of the preproghrelin or UAG is largely unknown although the acyltransferase that octanoylates ghrelin has recently been identified [71].

AG was identified first followed by UAG, then ghrelin was found to be involved in the regulation of food intake and energy expenditure, with AG the most potent peripheral orexigenic hormone known to date [64, 68, 70]. In animals and humans, AG has been shown to induce appetite and food intake differently to other anorectic gut peptides [68]. Despite these clear orexigenic effects of AG, the actual physiological role of UAG in the regulation of appetite is still a matter of debate [72]. Both forms of ghrelin also influence energy metabolism at the periphery, most likely influencing fat oxidation [73, 74]. Reduced cellular fat oxidation and promotion of adipogenesis both contribute to an increase in fat mass induced by AG and UAG [68, 75, 76].

Consistent with its effects on food intake and its involvement in energy balance, circulating ghrelin levels are negatively associated with body mass index in humans [68, 77, 78]. Several studies indicate that ghrelin hyposecretion in essential obesity is a functional impairment in response to body weight alteration [79]. Roux-en-Y gastric bypass has been shown to be associated with altered glucose kinetics and glucoregulatory hormone secretion likely secondary to the anatomic rearrangement of the foregut, with increased PYY and GLP-1 concentrations and decreased ghrelin levels [80, 81].

The majority of the data comes from studies that have exclusively analyzed total ghrelin levels. Recent findings have also shown that UAG is decreased in obesity, while there are no data for AG [82–86]. Ghrelin is implicated in the regulation of glucose homeostasis by modulating insulin secretion and action as described previously for other gut peptides with incretin action [64, 68]. During childhood, adolescence and adulthood, insulin modulates ghrelin levels through inhibition [64, 68, 87]. Ghrelin secretion is also affected by nutrients, namely carbohydrates, whereas the role of fats and proteins is not completely understood [64, 68, 88]. It is however noteworthy that the administration of mixed meals in children does not inhibit total ghrelin levels unlike adults, suggesting a modulation by age [64, 68, 89].

Ghrelin regulation and secretion during the fetal and neonatal periods is not fully understood yet but has been intensively investigated in the last seven years. Ghrelin is produced during fetal life, starting at 20 weeks post-conception and increases during the first years [86, 90–93]. These ghrelin

levels are directly produced by the fetus. Arterial and venous concentrations are similar and its expression in the placenta is almost absent during the third trimester [83, 94]. SGA newborns show higher ghrelin levels than AGA or LGA neonates [45, 86, 95]. Moreover, lower cord blood ghrelin levels are associated with slower weight gain from birth to 3 and 12 months of age, at least in breast fed infants [96, 97]; this evidence, however, does not definitely rule out a role of ghrelin in the control of somatotroph secretion but clearly suggests an involvement in both short-and long-term energy balance [94, 97]. Moreover, preterm AGA newborns have higher total ghrelin levels at birth [83, 94, 98, 99]. It is still not known whether ghrelin levels at birth in preterm neonates are independent or determined by gender, body weight and type of delivery [94]. What is clear is that ghrelin levels increase between birth and the fourth day of life and negatively correlate with weight and anthropometric parameters at that time [86, 94]. Ghrelin secretion is refractory to feeding in preterm AGA neonates during the first days of life, suggesting that it could represent an anabolic drive in newborns [94, 96]. However, ghrelin positively correlates with the enteral nutrition intake in both preterm and full-term babies on the second day after birth [100]. Blood ghrelin concentrations are affected by feeding, progressively increasing with prolonging fasting in full-term AGA infants from the first month of life [101].

Ghrelin secretion seems to be modulated by the type of enteral nutrition, suggesting a role of this peptide in gut development and maturation. At 4 months of age, formula fed infants have higher ghrelin and IGF-I levels without anthropometrical differences compared with breastfed ones [97]. At 6 months of age, ghrelin levels are higher in infants receiving a mixed diet of solids and milk compared with those exclusively milk fed [100]. It has been demonstrated that ghrelinaemia negatively correlated with weight gain at 12 months of age in breastfed but not in formula fed infants [97, 101]. These data suggest that a precocious derangement of ghrelin regulation could be linked to increased weight gain in children who are breastfed for less than 6 months [102]. Moreover, infant formula milks are devoid of many constituents, including ghrelin, which might influence gut maturation and fat accrual. Thus, both total ghrelin and AG increase in breast milk progressively during lactation during the first 6 months and their concentration in milk correlated positively with that in the plasma of breastfed infants [103].

Preliminary studies have also analyzed AG levels in newborns. AG is present in both fetal and neonatal circulation. AG and AG to total ghrelin ratio seem to be higher in venous cord blood, suggesting that the placenta is able to produce AG with a small part of it transferred to the fetal circulation [104]. AG is also higher in preterm and SGA newborns than full-term and AGA ones [104, 105]. Interestingly, umbilical cord AG concentration is lower than in maternal blood and the acylation process seems to be affected by cortisol partly through a placental role [99]. Any possible effect of AG on gut maturation has not yet been investigated.

43.2.4.2 Obestatin

In 2005, Zhang and coworkers identified a 23 amino acid peptide from a conserved region of preproghrelin sequence and named it obestatin, based on previous reported activities in animal models. Obestatin is amidated like GLP-1. It was initially characterized as the active ligand of the orphan receptor GPR39 [106] but more recently this has been debated [107, 108]. Obestatin, like ghrelin, is expressed in several tissues, including stomach, duodenum, pancreas, and brain [109]. Obestatin initially appeared to be a new regulator of appetite and body weight [108]. Subsequent studies, however, failed to reproduce obestatin's anorectic and anti-obesity effects and raised doubts about its existence simply as a ghrelin-associated peptide [110]. In contrast to other gut peptides, data regarding the modulation of insulin secretion are limited and controversial [107, 108]. However, preliminary *in vivo* studies observed that obestatin was reduced in subjects with type 2 diabetes, impaired glucose tolerance and in obesity, but increased in anorexia [111].

There have been some studies of the most recently identified gut peptide obestatin in pediatric subjects [89, 112], but there are no data relating to neonates.

43.2.5 Motilin

Motilin is a 22 amino acid peptide produced by the endocrine cells of the duodenum and jejunum. It binds and activates the receptor GPR38 through which it stimulates motility of digestive organs [113]. It regulates gastro-intestinal motility via migrating motor complex activity during phase III of contraction [114]. There are cyclical increases every 90-120 minutes during the interdigestive fasting periods [113, 114]. The mechanism by which motilin induces gut motility is still unclear. The gut motilin-containing cells and enteric neurons are only separated by a sheet of the basal lamina of mucosal epithelium. However, motilin receptor (GPR38) has not been demonstrated in the vagus nerve or nodose ganglion, although migrating motor complex activity is atropine sensitive and abolished by vagotomy [114, 115]. However, GPR38 is expressed in the brain, suggesting a role of motilin in feeding regulation [101]. It has to be emphasized that motilin and ghrelin precursors share almost 50% similarity in their sequences and, as a consequence, Tomasetto initially named it motilin-related peptide [116]. More recently, motilin has been included in the ghrelin peptide family. The GPR38 and GHS-R1a receptors are also part of the same family of G-protein coupled receptors with an overall sequence identity of the 53% [113]. Moreover, ghrelin and motilin are located in the same endocrine cells and in the same secretory granules at duodenum and jejunum level [117].

The role of motilin in gut development has been investigated during the neonatal period. Motilin secreting cells

appear at 8-11 weeks of gestation in the fetus, and at 12-15 weeks of gestation the distribution is similar to that seen in adulthood [118]. Motilin levels are higher in preterm and SGA infants after receiving enteral nutrition compared with levels in older children or adults [119–121]. Motilin is also present in human milk and its digestion in the stomach is retarded by milk itself, suggesting a biological role in the gut of newborns [101, 122]. Moreover, babies who have colic have higher motilin levels, which may have a role in this condition [123, 124]. Motilin-receptor antagonists could be a potential therapeutic agent for the treatment of colic when they become available for clinical trials [113].

43.2.6 Cholecystokinin (CCK)

CCK was the first gut hormone described as an inhibitor of food intake in rodents [125]. CCK is widely expressed at both central and peripheral levels, in particular in I-cells in the duodenum and jejunum mucosa [126]. CCK is detectable in the bloodstream in different forms, in particular CCK-8, CCK-33 and CCK-39 [16]. Two CCK receptor subtypes have been isolated: CCK_A receptor which is expressed in the vagus nerve, enteric neurons, pancreas and brain, and CCK_B expressed in the afferent vagus nerves, brain and stomach [126].

CCK has several activities on different tissues, including stimulation of pancreatic enzyme excretion and the induction of gallbladder contraction [10]. CCK has been shown to be a satiety signal, in particular in the short-term regulation of feeding behaviors [16, 126, 127]. It acts synergistically with leptin on the hypothalamic region [16, 126]. Its effect is reduced by vagotomy [16, 126, 127].

Peripheral CCK administration is followed by a rapid, but short-term effect on the inhibition of food intake, with an activity peak at 30 minutes [126]. High doses of CCK cause nausea, vomiting and taste aversion [128]. CCK mainly inhibits fat ingestion [129]. The inhibitory effect of CCK on food intake undergoes a functional desensitization after high dose administration as well as during continuous infusion [126]. When administered intermittently before meals, CCK maintains its anorectic effect, but a compensatory effect can be achieved by increasing the daily number of meals, however, there is only a mild effect on total food intake and body weight [128].

During the neonatal period, CCK has been shown to increase immediately after breastfeeding; this peak is followed by a decline 10 minutes later. There is a secondary rise 30 and 60 minutes later. The first increase of CCK levels may exert stimulatory effects on digestion while the secondary increase could have an anorectic effect [130]. Higher CCK levels correspond to a higher volume of ingested milk during breastfeeding [131]. CCK levels are negatively correlated with age in full-term infants [130]. Preterm infants show a similar increase in CCK concentration in cord blood 1 hour after delivery in both AGA and SGA babies [132, 133]. Plasma CCK concentrations appear to increase after nasogastric-tube feeding only in preterm neonates receiving kangaroo care and these results are still under investigation [134]. Furthermore, CCK prophylaxis for high-risk neonates seems to help with the prevention of parenteral nutrition-associated cholestasis [135]. However, the role of CCK on gut maturation has not yet been investigated.

43.3 Therapy and Treatments?

Bioactive peptides, hormones and factors in human milk and produced by the gut have been shown to stimulate gut development and function and to be modulated by nutrients. Most findings come from studies on preterm or SGA infants. However, there is a knowledge gap because many studies were performed 20 years ago and abandoned, while others have been linked to gut peptides discovered in recent years. Substances derived from these hormones might be used for the treatment of preterm newborns and colonic dysfunctions. However, further physiological and pharmaceutical studies are needed. In the future, more detailed studies will be needed to elaborate completely the functions of these peptides and the interaction of breast and formula milk and solids with the gut. Investigation of the signaling systems between neonates, mothers and nutrients could offer a physiological basis for appropriate nutritional support for preterm infants, as well as treatment options for necrotizing enterocolitis.

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44

Feeding the Term Infant: Human Milk and Formulas

Silvia Fanaro and Vittorio Vigi

44.1 Introduction

Breast milk represents the natural food for infants, and is universally recognized as the optimal feeding choice for every infant. The American Academy of Pediatrics (AAP), and the European Society for Pediatric Gastroenterolgy, Hepathology and Nutrition (ESPGHAN) recognize that feeding at the breast is the advisable way of supporting the natural growth and development of all infants [1, 2].

The health benefits of breastfeeding are particularly relevant under conditions of poor hygiene and low socio-economic status: in these instances receiving own mother's milk can be a matter of life or death [2]. In developed countries there is no precise evidence that breastfeeding has a substantial impact on infant mortality, even if it has a certain role in the reduction of gastrointestinal infections and acute otitis media [2]. The promotion and support of breastfeeding must be regarded as one of the fundamental role of the pediatrician. Unfortunately, the commitment of many people involved in the field of child care has not been sufficient in expanding the initiation and, above all, the duration of breastfeeding, which remains still unsatisfactory in many countries. Exclusive breastfeeding for the first six months of life is still not a common practice in both developed and developing countries. Pediatricians, who play a key role on child health, must promote and encourage breastfeeding, also taking in due account mothers' wishes.

Recommendations for the duration of exclusive breastfeeding are still debated. According to the WHO [3], human milk should represent the exclusive nutrient for full tem infants during the first 6 months of life. This recommendation is not generally fulfilled, because complementary foods are often introduced before the 6th month. However, breastfeeding should be continued through the first year of life and thereafter, as long as possible and wished [1].

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44.2 Breast Milk

Human milk is characterized by a peculiar species-specificity, with a composition that partially changes over time, during lactation, throughout the day and within a single feed (hind/fore milk), and that to some extent varies among different women. These variations are genetically determined, or induced by different lifestyles or dietary habits, mother's age and parity, as well as by the time of delivery. The most evident modifications take place in the first few days of lactation, when human milk changes from colostrum (0–5 days) to transitional (6–14 days), and then to mature milk (0.5–6 months; >6 months) [4].

Colostrum, the thick fluid produced during the first few days, is yellowish because of the high β-carotene content and contains more protein, fat-soluble vitamins and minerals, but less fats than the transitional or mature milk [5]. It is particularly rich in immunoglobulins and mononuclear cells [5]. During the transitional phase, from 7 to 10 days post partum to 2 weeks post partum, the composition gradually changes, with increasing concentrations of fats and lactose, into mature milk, whose composition remains relatively stable until weaning [5].

Maternal diet may influence milk composition, particularly for some components (fat quality and vitamins); although, it is important to point out that even extreme malnutrition does not induce consistent alterations in protein and lactose content, although it affects milk volume [5].

44.2.1 Nutritional Properties

Mature human milk is about 88% water and its osmolality is around 286 mOsm per liter [6].

The energy content of human milk varies during lactation and during a single feed, because it is mainly (~50%) determined by its fat content. Estimations for mature milk vary from 51 to 69 kcal/100 mL, but real values may be even

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

lower, as some components, mostly proteins and oligosaccharides escape digestion exerting non-nutritive effects [6].

Carbohydrates are the main component of human milk, and are mainly represented by lactose and oligosaccharides. Lactose is the prevalent nutrient in human milk, with an average concentration of 7 g/100 mL (6.7–7.6 g/100 mL) in mature milk [7]. Lactose is also the major determinant of milk osmolality and its concentration remains relatively stable once lactation is established [8]. Lactose, which is synthesized by the mammary gland, is a prompt source of galactose, which is essential for the production of galactolipids in the central nervous system (CNS) [5]. Oligosaccharides, the third largest component after lactose and lipids (2.4 g/100 mL in colostrum and 1.2 g/100 mL in mature milk), are non digestible carbohydrates which play a number of functional roles on intestinal health and host defenses, as will be more extensively described later [9].

Fat accounts for about 50% of the energy content of human milk and represents the second most abundant component after lactose, with a concentration of 1.5–2 g/100 mL in colostrum and 3.5–4.8 g/100 mL in mature milk [7]. The content is, however, extremely variable from woman to woman, during the course of lactation, during the day and, above all, during a single feed [10]. Lipids are a rather heterogeneous group of compounds, mainly represented by triglycerides (TGs, 98%) and in a small percentage by phospholipids, sphingolipids and cholesterol [7]. Lipids are secreted in a complex structure represented by the "milk fat globule", characterized by a core of TGs and cholesterol esthers, coated with bipolar substances such as phospholipids, proteins and bile salt-stimulated lipases. This unique system is effective in emulsifying milk fat and in improving the digestion and the absorption in the infant. Some of the substances included in the fat globule, such as lactadherin and MUC1, seem to play a role in host defense [11]. Fatty acids in human milk are for the main part estherified with glycerol and only in a small part are present in a free form [7]. While maternal diet does not influence the total fat content, which is however affected by body composition, it plays a significant role on the quality and on the proportion of the various fatty acids. More than 200 different fatty acids have been identified in human milk, represented by saturated (32–52%), cis-monounsaturated (30–50%), trans-monounsaturated (2.5–13.8%) and polyunsaturated fatty acids (11–27%) [6, 12]. The percentage of trans-fatty acids, as well as that of polyunsaturated acids, varies greatly worldwide, in relation to dietary habits. For example, the concentration of linoleic acid (18:2 n-6) may vary from 6% in omnivorous women to over 30% among vegan ones [13]. This aspect is even more evident for the n-3 eicosaenoid metabolite, docosahexaenoic acid (DHA, 22:6 n-3), whose content in human milk varies from 0.1% in women with vegan diets to 2.8% in Asiatic women with an high intake of fish and other marine foods [12]. The n-6 and n-3 fatty acids and their metabolites are essential for optimal growth and development of the brain and retina as for the synthesis of eicosanoids and leukotrienes [12].

Nitrogen content of human milk declines in the first two weeks of lactation from 300 mg/100 mL to 190 mg/100 mL, corresponding to a total protein content of about 1.2 g/100 mL (Kjeldahl's conversion factor = 6.25) [6]. Nearly 25% of this nitrogen is non-protein nitrogen (NPN), which includes urea, creatinine, sugar amines and free aminoacids; for this reason the real protein content is as low as 0.9 g/100 mL [6]. The milk protein fraction consists of casein and whey. The whey protein/casein ratio changes from 90:10 in early milk to 60:40 in mature milk and 50:50 in late lactation. Casein is made of four major families of protein: α, β, γ, and $\varkappa$ casein, among which β and $\varkappa$ are the most represented. Besides its function as a source of amino acids, casein serves as the precursor of several peptides with functional activities on intestinal motility and calcium absorption [14]. However, the whey fraction is the most characteristic and interesting component of human milk. It includes alpha-lactalbumin (αLA), lactoferrin, lysozyme, albumin, immunoglobulins, enzymes, hormones and growth factors [6]. The predominant whey protein in human milk is αLA, which provides a well-balanced supply of essential amino acids and is digested into peptides with antibacterial, immunostimulatory and antitumoral properties [15]. Some of the non-nutritional aspects of whey proteins of human milk will be further discussed later.

The vitamin content of human milk from well-nourished mothers is adequate to meet the needs of healthy infants with a few exceptions, represented by vitamin D and vitamin K [16]. In particular, vitamin D, whose production is almost exclusively dependent from sunlight exposure, is extremely low in human milk (0.4–4 IU/100 mL; 0.01–0.1 μg/100 mL) [17]. Thus, even breast fed infants born to mothers with normal vitamin D status are at risk of vitamin D deficiency in the first months of life. For this reason the American Academy of Pediatrics (AAP) recommends supplementing all breastfed infants with 400 IU/day of vitamin D from the first few days of life and to continue supplementation throughout childhood [18]. Vitamin K, which is essential for the synthesis and function of blood clotting factors and the structure of bones, is present in human milk at marginal concentrations. Measurements with advanced techniques have confirmed rather low levels, corresponding to 0.2–0.7 μg/100 mL [5, 19]. This is the reason why breastfed infants, receiving an oral dose vitamin K only at birth, undergo a serious risk of late-onset severe hemorrhagic disease. Although, even infants who had received intramuscular vitamin K at birth present low serum levels of vitamin K, with detectable proteins induced by vitamin K absent (PIVKAs) [20]. Notwithstanding this, very few cases of late hemorrhagic disease have been reported in breastfed infants who had received an intramuscular injection of vitamin K soon after birth. The AAP still recommends a single intramuscular dose of 0.5–1 mg to be given to all newborns [21]. At the same time, the AAP recommends that additional research should be conducted to test the safety and bioavailability and optimal dosing regimens of oral formulation of vitamin K [21]. Some authors and pediatric societies have proposed

to continue supplementation with physiological oral daily doses of 25 μg, recently revised to 50 μg, for the first three months of life in every breast fed infant [22, 23]. Human milk supplies adequate levels of the other fat-soluble and water-soluble vitamins with the exception of vitamin B12 in the case of mothers with vegan diet or with gastric by-pass [6].

Minerals are generally independent of maternal mineral status and do not significantly respond to supplementation [24]. In general, the concentration of macro-minerals, in particular calcium and phosphorus, is particularly low, but at the same time their bioavailability is particularly high [25]. With the exception of magnesium, all macro-minerals decline progressively during lactation [5]. The concentration of iron, zinc, and other trace elements also declines during lactation to very low levels; however, their bioavailability is much higher (20-50%) than that of cows' milk or infant formulas [26]. For this reason, breast milk provides sufficient intakes of trace elements to healthy term infants until the sixth month of life [27].

44.2.2 Non-Nutritional Properties

The supremacy of breast milk in the nutrition of the human infant results from both unique nutritional aspects and several valuable protective properties on the gastrointestinal function, the resistance to infection, as well as on the immunity, the neurodevelopment and the general well-being. Epidemiological data have clearly evidenced the preventive effect of human milk on infections (gastroenteritis, upper and lower respiratory tract illnesses, urinary tract infections, and sepsis) [28]. Although the impact of breastfeeding on the development of allergies remains controversial, some data indicate a transient, protective effect of exclusive breastfeeding, for at least 4 months, on atopic eczema, wheezing, and asthma in the first years of life [29, 30]. Other advantages on immune system regulation that have been investigated include the prevention of type 1 diabetes, celiac disease and inflammatory bowel disease, in particular Crohn's disease [2]. Breast milk may also exert a biological advantage on adult diseases, such as obesity, hypertension, hypercholesterolemia, cardiovascular disease and type 2 diabetes [2, 31–33]. The modulation and stimulation of the immune system could also partly explain the preventing effect of human milk on various malignant diseases, such as lymphocytic and myelogenous leukemia and breast cancer [34, 35]. However, the evidence of this preventive effect is at the moment rather weak [2]. Many studies have been performed on the effects of breastfeeding on neuro-development; most of them have evidenced an advantage, even if limited, on cognitive function, after also adjusting for potential confounders [2].

As a general rule, human milk is considered the cheapest and easiest way of feeding an infant. The economic advantages of breastfeeding are not limited in saving the costs of formula milk and go well beyond the familiar interest. The reduction of episodes of illnesses, fewer hospital admissions, medical visits and drug use, together with fewer absences of parents from work, associated with the reduction of chronic diseases in childhood and adulthood, represent an evident social and economic benefit.

44.2.3 Bioactive Components of Human Milk

A variety of heterogeneous agents with different beneficial activities on infants' health have been identified in human milk (Table 44.1) [36]. The value of these substances, pertaining to protein, lipid and carbohydrate fractions, is far beyond the nutritional aspect, which is frequently negligible. For reasons of space, our attention will be focused on few elements whose benefits are well recognized.

44.2.3.1 Oligosaccharides

Oligosaccharides (OS), carbohydrates made up of 3–9 monomer units, are synthesized in the mammary gland by specific enzymes (glycosyltransferases), by adding monosaccharide units (galactose, N-acetyl-glucosamine, fucose, sialic acid) to a molecule of lactose, forming either linear or branched structures [37]. More than 200 different forms of acidic or neutral oligosaccharides have been described, and their composition is related to maternal genetic expression of different fucosyltransferases [9, 37]. Oligosaccharides are important prebiotics and may represent a relevant component of the so-called "bifidus factor" of human milk. They resist the enzymatic digestion in the gastrointestinal tract, thus reaching the colon intact. At this level OS are mainly utilized in bacterial fermentation, leading to the production of short-chain fatty acids (acetate, propionate, and butyrate) and gases (hydrogen, carbon dioxide, and methane), and so selectively promoting a bifidogenic flora [38, 39]. The intestinal flora of breastfed infants is, in fact, typically characterized by a prevalence of bifidobacteria, while the growth of several other organisms is somehow reduced [40]. Lactic acid bacteria, in particular bifidobacteria, are widely recognized to positively influence human health, both at local and systemic levels, and are certainly involved in many biological advantages that breastfed infants have over formula fed counterparts [39].

44.2.3.2 Lactoferrin

Lactoferrin (LF) is an 80 kDa, natural defense iron-binding glycoprotein, which is present in several exocrine secretions, including tears, nasal and bronchial mucus, saliva, intestinal and genital secretions [41]. Its concentration in colostrum is up to 600 mg/100 mL and progressively decreases in mature milk to 140 mg/100 mL, while in standard infant formulas it is 10 fold lower [42]. Thus, in human milk lactoferrin accounts for about 10–15% of the total protein content [43].

Table 44.1 Bioactive factors of human milk

Adaptive immunity compounds	Immunoglobulins sIgA (11S), 7S IgA, IgG, IgM, IgE, IgD, free secretory component, antiidiotypes
Innate immunity agents	Complement, chemotactic factors, properdin factors, interferon, α-fetoprotein, antistaphylococci factors, mannose binding lectin, β-defensin-1, antiadherence substances (oligosaccharides, mucins, lactadherin, glycolipids and glycosaminoglycans, ϰ-casein), milk fat globule, hormones and growth factors (prolactin, cortisol, insulin, thyroxin, prostaglandins, vascular-endothelial growth factor, nerve growth factor, TGF, erythropoietin), antiviral factors (fatty acids and monoglycerides), migration inhibition factor, α-lactalbumin
Cytokines, chemokines, and receptors	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12, IL-13, IL-16, IL-18, IFNγ, TNFα, G-CSF, M-CSF, GM-CSF, GROα, monocyte chemotactic protein-1, TGFβ1 and -2, sCD14, Toll-like receptor, sFas, sFasL
Antiinflammatory factors	IL-10, TGFβ2, glucocorticoids, antioxidants (α-tocopherol, β-carotene, lutein, vitamin E, catalase, glutathione peroxidase), lactoferrin, IL-1Ra, soluble TNFα receptors I and II, CD59
Prebiotics	"Bifidus factor", oligosaccharides, lactoferrin
Histocompatibility antigens	
Carrier proteins	Lactoferrin, transferrin, vitamin B-12 binding protein, steroid binding protein
Enzymes	Lysozyme, lipoproteinlipase, leukocyte enzymes, antiproteases, platelet-activating factor-acetyl-hydrolase
Others	Nucleotides, long-chain polyunsaturated fatty acids
Cellular	
Total counts	Colostrum, $1–3 \times 10^9$/L; mature milk, ~1×10^8/L
Cell types	Macrophages, ~60%; neutrophils, ~25%; lymphocytes, ~10%; epithelial cells

Modified from [36].

Lactoferrin has a marked bacteriostatic and bactericidal effect on a wide range of microorganisms, not only because of the iron deprivation of the pathogens, as believed in the past, but also because of its metabolite lactoferricin, a potent bactericidal peptide [44, 45]. In addition, lactoferrin shows a serine protease activity, which degrades the bacterial structures necessary for the attachment and the invasion of the bacteria to the mucosa [46]. Other valuable properties of lactoferrin are antiviral and fungistatic activities. Another precious effect of lactoferrin is the promotion of a bifidus flora. In fact, lactoferrin resists digestion by pepsin, trypsin and chymotrypsin and shows a bifidogenicity 100 fold stronger than N-acetylglucosamine [45, 47]. In addition, lactoferrin presents an antineoplastic action *in vitro* and a dual effect on immunity response, mostly anti-inflammatory [44, 48, 49].

44.2.4 Growth of Breastfed Infants

Several studies published in the last 20 years have repeatedly indicated different growth patterns in breastfed and formula-fed babies; the former show, in fact, a reduced rate of weight and length accretion from the 3rd–4th month to the 12th month of life [50]. In the past years this downward trend has somewhat worried some pediatricians and health care providers, leading to the erroneous conclusion that growth faltering was connected to inadequate breast milk intake or even to breast milk inadequacy. This conclusion has often led to the premature introduction of complementary foods and/or to the use of supplementary milk formulas. New growth charts based on thousands of breastfed children from 6 different countries were released in 2006 by the WHO [51]. The subjects examined, aged 0–5 years, were living in socio-economic conditions and in environments supportive of growth [51]. All the children studied were breastfed for at least 12 months [51]. These new curves reflect the growth when the subjects are fed optimally to achieve their genetic growth potential [52]. The use of these new standards avoids the risk of unnecessary formula supplementations. There is no evidence that individuals who are fed at the breast becomes shorter adults, but there is growing evidence that they may be protected from obesity later in life [52].

44.2.5 The Practice of Breastfeeding

According to the indication of AAP, in the first few weeks an infant may require 8 to 12 feedings a day, avoiding night intervals longer than 4 hours [1]. The postnatal weight loss should never be more than 8-10% and the birth weight should be regained within the 14th day of life [1]. The adequacy of milk intake may be judged on the basis of several indicators such as the number of wet diapers and the number, quantity and color of stools. During the first weeks of life the infant should produce six wet diapers/day, with yellow pale urine, and almost one yellow stool after each feeding [1]. Criteria are available when an intervention is necessary because the baby is undernourished [53]. Any infant who is not gaining a minimum of 20 g/day in the first months of life should be closely scrutinized and evaluated.

44.3 Infant Formulas

Infant formulas have tended for many years to be as close as possible to human milk, approximating its macro and micro

nutrient composition [54]. Although, the consistent variations of breast milk composition during lactation, within the day and within a single feed (hind and fore milk) have indicated that the figures relative to the composition of human milk cannot be taken as absolute values, but may only represent a general guide for the biochemical formulation of human milk substitutes. Thus, in the last few years it has been generally accepted the concept that milk formulas should approach the results obtained by breast milk, more or less independently by a strict adherence to its chemical composition. In other words, the adequacy of an infant formula is determined by a comparison of its effects on physiological (e.g., growth patterns), biochemical (e.g., plasma markers) and functional outcomes (e.g., immune responses) in infants fed formulas with those found in healthy breastfed infants [55]. Cow milk based infant formulas are the only acceptable alternative when human milk is not available. Although, cow milk has an unbalanced fat composition and an excessive concentration of proteins and minerals; for these reasons it represents only the matrix, which must be largely modified and adapted to the needs of human infants. New formulas are continually developed and new ingredients are added to existing formulas, as long as new components and biological activities of human milk are identified. At present, our scarce knowledge of the real functions of many substances present in human milk, such as hormones, immune and growth factors, enzymes and viable cells, makes it practically impossible to add them to current milk formulas [56]. Although, positive results of recent investigations have made the introduction of new important substances possible, such as Lc-Pufas, nucleotides, oligosaccharides, lactoferrin and the favorable modification of the protein fraction.

At present, human milk substitutes can be considered as the most regulated and controlled commercially available foods. The European Community has issued a Commission Directive on Infant Formulas, last amended in 2006, which has been implemented by member states [57]. In the United States the infant formula Act of 1980 has set up quality control procedures, minimum requirements and maximum permissible levels of nutrients contained in milk formulas [58]. A milk formula has been defined as "a food which purports to be or is represented for a special dietary use solely as a food for infants by reason of its simulation of human milk or its suitability as a complete or partial substitute for human milk" [58].

One aspect that deserves particular attention is the safety of preparation, storage and handling of infant formulas, especially if in powdered form. In fact, there is a serious risk of contamination of milk powder with *Cronobacter* (*Enterobacter*) *sakazakii*, which represents a possible etiologic agent of severe infections and sepsis in infants, especially if younger than 3 months, preterm, and immunocompromised [59–62]. For this reason, special attention should be paid giving parents appropriate instructions concerning the preparation of powdered milk formulas, as recommended by WHO: wash and sterilize bottles, wash and dry hands properly, use hot water (70–80°C), then add powder, quickly cool to 40°C to safely feed the infant, and finally discard the remaining [63]. If parents need to prepare feeds in advance for later use, reconstituted milk formula, as previously indicated, should be rapidly cooled and placed in a refrigerator (temperature ≤ 4°C) for a maximum of 24 hours [63]. In care settings the use of liquid milk formulas, as well as of sterile disposable bottles is obviously strongly recommended; it is advisable to reconstitute the milk immediately before feeding the infant.

44.3.1 Lipids

Fats in infant formulas provide 40–50% of the total daily energy intake of an infant. Lipids currently employed in standard infant formulas are of vegetable origins. A great deal of discussion has been aroused over the last 20 years by the addition of long-chain polyunsaturated fatty acids (Lc-Pufa). The interest of Lc-Pufas is directly linked to the recognition of their important role in the development and maturation of the infant nervous system. DHA is preferentially accumulated in the membranes of photoreceptors, cells of the retina, and in neurons during the last trimester of pregnancy and the accumulation continues along the first two years of postnatal life [12]. Other Lc-Pufas, i.e., arachidonic acid (ARA, 20:4 n-6) and eicosapentaenoic acid (EPA, 20:5 n-3), are precursors of important regulatory molecules, such as prostaglandins, leukotrienes, and thromboxanes [12]. Term infants are able to synthesize Lc-Pufas from precursors, but endogenous synthesis may be insufficient to cover the infant's DHA and ARA requirements [64]. During the first months of life breastfed infants receive preformed DHA and ARA. Feeding a non-supplemented infant formula leads to significantly lower levels of the DHA contained in plasma lipids, in erythrocyte membrane phospholipids, and in the cerebral cortex [64]. Data concerning the importance of offering preformed DHA to the infant have shown variable results on visual function and neurodevelopment outcomes [65, 66]. However, a number of papers provide a strong rationale for supplementing formulas with DHA and ARA. For this reason the International Expert Group (IEG) of ESPGHAN, among other scientific organizations, supports their optional addition in infant formulas, specifying that the DHA content should not exceed 0.5% of total fat intake, that ARA content should be at least the same concentration as DHA, and that the EPA content should not exceed the DHA content [55]. The EU Commission Directive established the minimum and maximum levels of the different fatty acids:

- linoleic acid (300–1200 mg/100 kcal)
- alpha-linolenic acid (not less than 50 mg/100 kcal)
- linoleic/alpha-linolenic acid ratio (5–15)
- n-3 LCP (not more than 1% total fat, DHA should not exceed total n-3 LCP content)
- n-6 LCP (not more than 2% of total fat, for ARA not more than 1% of total fat)
- EPA not more than DHA [57].

44.3.2 Proteins

In the past years formula fed infants consumed 60–70% more protein than breastfed infants and their plasma aminoacidogram was markedly different from those found in breastfed infants. Even though it was demonstrated that a milk formula with a whey-casein ratio of 50/50 produced an amino acid pattern more similar to that of human milk, the amino acid profile remained substantially different from that of breastfed infants [67]. There has been considerable discussion regarding the optimal concentration of protein in infant formula and the risks of excessive protein intake. Even though there was strong motivation to develop formulas that ensure a protein composition closer in quantity and quality to human milk, practical problems were serious obstacles for this accomplishment. The protein content of infant formulas are not species-specific, so that if they are reduced below certain limits, several essential amino acids could fall below the desired levels. In the last few years the protein content of formulas has been substantially modified and reduced, employing two approaches: removal of the whey protein glycomacropeptide, thus reducing plasma threonine concentration, and addition of alpha-lactalbumin, a tryptophan rich protein, balancing the entire amino acid profile [68]. Infants fed a low protein infant formula enriched with alpha-lactoalbumin have serum amino acid patterns near those of breastfed infants [68]. Besides its positive effect on aminoacidogram, αLA is believed to exert several valuable effects on health, such as antimicrobial activity, enhanced immune function, prebiotic function, increased trace element absorption and anti-tumoral activity [45, 69]. These interventions may overcome the problem associated with high protein intakes, allowing normal weight gain, without suboptimal amino acid levels [70]. The EU Commission Directive stated that the protein content of infant formulas manufactured from cow milk proteins must range from 1.8 g/100 kcal to 3 g/100 kcal, specifying that the infant formula must provide an available quantity of all indispensable and conditionally indispensable amino acids at least equal to that contained in breast milk [57]. Thus, it is possible to reduce the protein concentration in formulas to 13 g/L, provided that high-quality protein sources are used. The IEG is doubtful regarding the adequacy of the minimum allowed protein level of formulas, in terms of growth and amino acids supplied and it reaffirms the necessity of measuring the true protein content of a formula utilizing the conversion factor of 6.25 ([Total N-NPN] × 6.25) [55].

Lactoferrin, (LF) is a multi-functional whey protein that represents 10–20% of the total protein content of human milk [43]. Lactoferrin has shown important activities both *in vitro* and *in vivo*, such as bacteriostatic and bactericidal, antiviral and fungistatic effects, bifidogenic activity, anti-oxidant and anti-neoplastic action and a dual effect on the immune response, mostly anti-inflammatory [44–49, 71, 72]. For these reasons there is a growing interest toward the clinical advantages deriving from its administration both to infants and adults. In particular, there is a growing consensus toward the addition of lactoferrin to infant formulas, as already done in Japan for many years. In addition, bovine lactoferrin (BLF) is characterized by a high (77%) amino acid homology with human lactoferrin (HLF) and this aspect may be helpful in improving the amino acid pattern of formula-fed infants. At the moment, in Italy, only two manufacturers have introduced LF in preterm formulas, while there is only one infant formula supplemented with a rather low concentration (14 mg/100 mL) of lactoferrin. At present, there is no official recommendation concerning the addition of lactoferrin to infant formulas.

44.3.3 Carbohydrates

Carbohydrates represent the most important source of energy for the infant after lipids. Minimum and maximum carbohydrate content are established at 9.0 and 14.0 g/100 kcal, corresponding to 36–56% of the total energy of infant formulas [55]. Lactose represents the main digestible carbohydrate milk formulas as well. No maximum level specific for lactose has been set, whereas the minimum content is set to 4.5 g/100 kcal [57]. Free glucose, even though it is highly absorbable, is not routinely added to formulas, because of its heavy effect on osmolality and because of the possible unpleasant Maillard reaction with proteins. The addition to infant formulas of sucrose is not recommended by the IEG of ESPGHAN because of possible severe reactions in young infants affected by hereditary fructose intolerance [55]. Although, both glucose and sucrose may be added to infant formulas manufactured from protein hydrolysates [57]. Maltose and malto-dextrins, generally produced by controlled hydrolysis of corn starch, are sometimes employed to improve the gastro-intestinal tolerance of milk formulas, without increasing the osmolality load. Starch may be added to infant formulas up to 2 g/100 mL (30% of total carbohydrates) [55, 57].

Oligosaccharides (OS), which are carbohydrates with a degree of polymerization (DP) ranging from 2 to 60 monomers, are non-digestible by human or animal digestive systems. As previously said, they represent the third largest component after lactose and lipids of human milk. Technologically, they can be extracted from natural sources or derived from hydrolysis of natural polymers or synthesized from monomers and/or small oligosaccharides. Different OS have been investigated for their prebiotic effect in infancy, such as neutral and acidic galacto-oligosaccharides (GOS), short- and long chain fructo-oligosaccharides (scFOS), inulin, and combinations such as a mixture of these substances [73, 74]. A recent systematic review of published randomized clinical trials on the effectiveness of prebiotic supplementation in term infants, has shown that formulas supplemented with prebiotics are well tolerated and result in various short-term beneficial effects, including increased stool colony counts of bifidobacteria and lactobacilli, decreased counts of pathogenic enteric bacteria, more

acidic stools, and softer and frequent stools, without adversely affecting weight gain [75, 76]. These are certainly interesting results; however, the importance of prebiotics in the defense against pathogens and in the modulation of the immune responses needs further experimental evidence and scientific substantiation. The effects of oligosaccharides on the prevention of atopic diseases are limited and still debated [77–79].

The EU Directive on infant formulae expresses no objection to the addition of a mixture of galacto- and fructo-oligosaccharides to infant formulas up to a maximum concentration of 0.8 g/100 mL, and of other oligosaccharides whose favorable effects in infancy have been proven with scientific criteria [57].

In conclusion, as observed several years ago by an eminent scientist and one of the greatest authority in infant nutrition, Samuel J. Fomon, milk formula "…is not as good as breast milk, and can never be breast milk. But it can be a healthy alternative and is always being improved".

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45

Nutritional Recommendations for the Very Low Birth Weight Newborn

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45.1 Introduction

To ensure uninterrupted growth and development of the prematurely born infant, all nutrients must be provided in the necessary amounts at all times. Delivery of nutrients meets with a variety of obstacles in very low birth weight (VLBW) infants, which is why VLBW infants frequently receive less than the required amounts of nutrients. As a consequence they fail to grow like they would have in utero and, more importantly, suffer impairment of their neurocognitive development. The main obstacles to delivery of nutrients historically have been concerns regarding presumed risks attendant to the administration of nutrients. Recent years have seen a gradual dissipation of these concerns as scientific evidence of the safety of providing nutrients has accumulated. This reassessment of the risks of nutrient administration has nearly been completed in the area of parenteral nutrition but is an ongoing process in the area of enteral nutrition. The more realistic assessment of risks, together with a desire to prevent postnatal growth failure and thereby minimize the risk of impaired neurocognitive impairment has led to more aggressive regimens of nutrient administration.

There is principal agreement that postnatal growth of the premature infant should emulate that of the fetus in utero. Such "normal" growth would be proof of the absence of substantive nutrient deficiency and would provide assurance of unimpaired neurocognitive development. The American Academy of Pediatrics [1] recommends that "postnatal growth that approximates the in utero growth of a normal fetus of the same postconception age" should be the basis for estimating nutrient requirements. It is recognized that birth engenders a permanent contraction of body water spaces, amounting to about 10–15% of extracellular fluid space. However, there are no known reasons why other body components should not be accumulating in the premature infant as they would have in the fetus. The fetal model is therefore appropriate for the estimation of nutrient intakes needed for normal growth and development.

Nutrient needs of the premature infant have been determined principally by two methods, the factorial method and the empirical method. Although both methods are ultimately based on the fetal model, they use very different approaches. In the factorial approach, fetal accretion of body components is used as the starting point. After correction for inevitable losses, inefficiency of conversion of nutrients into body components (in the case of protein) and absorptive inefficiency, the required intake for a nutrient is obtained. An important advantage of this approach is that it yields requirements for all nutrients for which fetal accumulation is known, which includes, besides protein and energy, all major minerals and most trace minerals. The factorial approach can also provide needs specifically for parenteral administration of nutrients. In the empirical approach, the intake of a nutrient is experimentally manipulated and a response, such as weight gain or, in the case of protein, nitrogen balance is observed. This approach has been applied to protein end energy and yields energy and/or protein intakes that produce weight gain or protein accretion comparable to the fetus. Estimation of nutrient needs for catch-up growth is possible with the factorial as well as the empirical approach.

45.2 Protein Requirements Determined by the Factorial Approach

The factorial approach derives nutrient requirements as the sum of two (in the case of parenteral requirements) or three components (in the case of enteral requirements). The largest component, and the component that changes most with body size, is nutrient accretion. The other components are inevitable losses and, in the case of enteral requirements, efficiency of nutrient absorption.

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Table 45.1 Estimated nutrient intakes needed to achieve fetal weight gain

Body weight (g)	500–700	700–900	900–1200	1200–1500	1500–1800	1800–2200
Fetal weight gain (g/d)	13	16	20	24	26	29
(g/kg/d)	21	20	19	18	16	14
Protein (g/kg/d)						
Loss	1.0	1.0	1.0	1.0	1.0	1.0
Growth (accretion)	2.5	2.5	2.5	2.4	2.2	2.0
Required intake						
Parenteral	3.5	3.5	3.5	3.4	3.2	3.0
Enteral	4.0	4.0	4.0	3.9	3.6	3.4
Energy (kcal/kg/d)						
Loss	60	60	65	70	70	70
Resting expenditure	45	45	50	50	50	50
Other expenditure	15	15	15	20	20	20
Growth (accretion)	29	32	36	38	39	41
Required intake						
Parenteral	89	92	101	108	109	111
Enteral	105	108	119	127	128	131
Protein/Energy (g/100 kcal)						
Parenteral	3.9	3.8	3.5	3.1	2.9	2.7
Enteral	3.8	3.7	3.4	3.1	2.8	2.6

Nutrient accretion is derived from body composition of the fetus. Going back to the 19th century, the chemical composition of a sizable number of term and preterm infants, who were stillborn or died soon after birth, have been reported by a number of investigators. Sparks [2] and Forbes [3, 4] have provided comprehensive summaries of the chemical data derived from whole body chemical analyses of over 160 infants. The data permit derivation of fetal accretion rates of the various body components [2–4].

Utilizing a different approach, Ziegler et al [5] used select data for the construction of a "reference fetus" and similarly derived fetal accretion rates. Accretion rates by the different approaches are similar and the fetal accretion rates in Table 45.1 represent a composite of the different approaches [2–5] in combination with contemporary fetal growth data [6]. Fetal accretion rates shown in Table 45.1 are corrected for presumed inefficiency (90%) of the conversion of dietary protein to body protein. Energy accretion values include the energy cost of growth, estimated by Micheli et al [7] at 10 kcal/kg/d.

Inevitable losses of protein (nitrogen) through desquamation of skin were assumed to be 27 mg/kg/d [8] and urinary losses in the form of urea to be 133 mg/kg/d [9, 10]. Energy losses in the form of resting energy expenditure were assumed to be 45 kcal/kg/d in infants <900 g and 50 kcal/kg/d in larger infants, and other expenditures, e.g., for occasional cold exposure and physical activity, were assumed to be 15 kcal/kg/d in infants <1200 g and 20 kcal/kg/d in larger infants [11, 12]. The requirements for parenterally administered protein and energy (Table 45.1) are calculated as the sum of accretion plus inevitable losses. Enteral requirements are derived from parenteral requirements by application of corrections for percentage absorption, assumed to be 88% for protein and 85% for energy.

Absolute fetal weight gain (g/d) increases with increasing body size, but fractional fetal weight gain (g/kg/d) decreases markedly with increasing body size. In spite of this, the rate of protein accretion remains constant up to a weight of 1200 g. This is so because the protein content of fat-free body mass increases with increasing body size/age, offsetting the effect of the decrease in fractional growth rate. Energy accretion, on the other hand, increases with increasing body weight due to a marked increase in fetal body fat content. As the accumulation of body fat is not an absolute necessity, lesser energy intakes do not limit growth of lean body mass as long as they are greater than 100 kcal/kg/d [7].

45.3 Protein Requirements Determined by the Empirical Approach

The empirical approach utilizes feedings (formulas or human milk) that provide precisely known intakes of energy and protein, with growth and/or nitrogen balance as outcomes. Because of the fragility of extremely immature infants, the necessary studies have been performed for the most part with infants weighing >1200 g. As requirements are strongly influenced by body size, estimates by the empirical approach are generally applicable only to infants weighing >1200 g. Data published before 1986 [13], showed that weight gain (g/day) increased with increasing protein intake all the way to the highest intake studied (3.6 g/kg/day) Weight gain did not seem to be influenced by energy intake. The high protein

Table 45.2 Protein requirements by the empirical method and recommended intakes for premature infants

	Weight <1200 g		Weight >1200 g	
	g/kg/d	g/100 kcal	g/kg/d	g/100 kcal
Ziegler (Table 45.1)	4.0	3.7	3.6	2.8
Kashyap & Heird [14–16]	–	–	3.0	2.5
Denne [19]	3.5–4.0	–	3.0	–
Klein et al [18]	3.4–4.3	2.5–3.6	3.4–4.3	2.5–3.6
Rigo [17]	3.8–4.2	3.3	3.4–3.6	2.8

intake of 3.6 g/kg/d was shown to produce a weight gain of about 30 g/day, which was more than the weight gain of the fetus and therefore represented catch-up growth. Kashyap, Heird and collegues [14–16] performed a series of growth and metabolic balance studies with feedings (human milk, formulas) that varied in protein and energy content. They used the data to derive equations predicting protein and energy intakes necessary to duplicate fetal weight gain. The authors estimated that protein intake necessary for infants with birth weight greater than 1200 g to duplicate fetal weight gain was about 3.0 g/kg/day (Table 45.2). Using a variety of endpoints, including growth, body composition and nitrogen balance, Rigo [17] estimated the protein requirements ("advisable recommendation") of infants born at 26–30 weeks gestation (corresponding to weight of about 800 to 1500 g) at 3.8–4.2 g/kg/d (3.3 g/100 kcal) and those of infants born at 30–36 weeks gestation (corresponding to weight of 1500 to 2700 g) at 3.4–3.6 g/kg/d (2.8 g/100 kcal) (Table 45.2).

45.4 Recommended Protein Intakes

Based on a review of published studies, the Life Sciences Research Office (LSRO) [18] concluded that the minimum protein intake of premature infants (weight not specified) was 3.4 g/kg/d with a protein/energy ratio of 2.5 g/100 kcal at the maximum energy intake of 135 kcal/kg/d. The LSRO also concluded that a protein intake of 4.3 g/kg/d (with protein/energy ratio of 3.6 g/100 kcal) was without adverse consequences, whereas intakes of 5. 0 g/kg/d or higher were likely to be associated with undesirable consequences. Denne [19] provided recommended intakes based mostly on the factorial approach.

Table 45.2 provides a summary of the different estimates of requirements and of recommendations for infants weighing less than 1200 g and those weighing more than 1200 g. It is apparent that there is reasonably close agreement among the different estimates in spite of differences in methods and endpoints used.

45.5 Requirements for Energy and Other Nutrients

Estimates of energy requirements are available by the factorial method (Table 45.1). However, estimates by the empirical method are considered more relevant with regard to energy. Empirical estimates [7] indicate that energy needs are 90–100 kcal/kg/day. Energy taken in excess of this amount is used for storage in adipose tissue.

Requirements for major minerals and electrolytes derived by the factorial method are summarized in Table 45.3. Because there is considerable uncertainty regarding the minimal urinary losses of electrolytes and phosphorus, and because there is large variation of the efficiency of intestinal absorption of calcium, the required intakes shown in Table 45.2 are somewhat uncertain. Since accumulation of less than the fetal amounts of bone mineral (Ca, P) seems to be compatible with reasonable bone health, there is even uncertainty with regard to the minimum amount of Ca and P that must be accrued. Nevertheless, fetal accretion rates are considered the norm and deviations from them need to be justified. Table 45.4 summarizes the minimum mineral and vitamin content of feedings for premature infants as recommended by the LSRO [18].

Table 45.3 Requirements for major minerals and electrolytes determined by the factorial method (all values per kg/day)

	500–1000 g		1000–1500 g		1500–2000 g	
	Accretion	Required intake	Accretion	Required intake	Accretion	Required intake
Ca (mg)	102	184	99	178	96	173
P (mg)	66	126	65	124	63	120
Mg (mg)	2.8	6.9	2.7	6.7	2.5	6.4
Na (meq)	1.54	3.3	1.37	3.0	1.06	2.6
K (meq)	0.78	2.4	0.72	2.3	0.63	2.2
Cl (meq)	2.26	2.8	0.99	2.7	0.74	2.5

Table 45.4 Minimum mineral and vitamin content of feedings for preterm infants

	Amount per 100 kcal
Minerals	
Na (mg)	39
K (mg)	60
Cl (mg)	60
Ca (mg)	123
P (mg)	82
Mg (mg)	6.8
Fe (mg)	1.7
Zn (mg)	1.1
Cu (μg)	100
Mn (μg)	6.3
I (μg)	6
Se (μg)	1.8
Vitamins	
A (μg RE)	204
D (IU)	75
E (mg α-TE)	2
K (μg)	4
B1 (μg)	30
B2 (μg)	80
Niacin (μg)	550
B6 (μg)	30
B12	0.08
Folic acid (μg)	30
Pantothenic acid (μg)	300
Biotin (μg)	1
C (mg)	8.3

45.6 Catch-up Growth

Growth restriction, whether occurring before birth (intrauterine) or postnatally, is associated with impaired neurocognitive development. Although postnatal growth restriction may lead to lower cardiovascular and metabolic risks later in life [20], the overriding concern is with neurocognitive development. Therefore, in order to minimize the adverse effects of growth restriction, efforts should be made to allow infants to make up any growth deficit as soon as possible. Accelerated growth following a period of growth restriction is referred to as catch-up growth.

Most infants are capable of catch-up growth, and catch-up growth will occur if the requisite amounts of nutrients are provided. Nutrient needs for catch-up growth are above and beyond the needs for growth like the fetus. Since most premature infants will have incurred some growth deficit during the immediate postnatal period, it is advisable to provide all VLBW infants with sufficient nutrients for making up the growth deficit. Attempts to estimate nutrient needs of growth-restricted infants and needs for catch-up growth have been made [21].

Table 45.5 presents examples of nutrient requirements of hypothetical growth restricted infants. The first column gives nutrient requirements of a normally-grown 26-week gestation infant weighing 900 g. The next column shows requirements of a growth restricted infant of similar weight and growing at the same rate as the normally grown infant. Because the lean body mass of this infant has a more mature composition (lower water content), the infant's metabolic rate is higher and accordingly its energy requirement is higher than that of the normally grown infant. The hypothetical infant in the far right column is also growth restricted but is assumed to undergo catch-up growth. As shown, this increases the requirements for energy and, especially for protein, by a considerable margin. Unless these needs are met, the infant will not realize catch-up growth.

Table 45.5 Nutrient requirements of growth restricted VLBW infants without and with catch-up growth

Gestational age	26 wk	30 wk	30 wk
Weight (g)	900	900	900
Weight status	NG	GR	GR
Catch-up growth	no	no	yes
Weight gain (g/d)	20	20	34
Required intake			
Protein (g/kg/d)	4.0	4.0	4.9
Energy (kcal/kg/d)	119	126	141
Protein/energy (g/100 kcal)	3.4	3.2	3.5

NG normally grown, *GR* growth restricted.

45.7 Nutrient Delivery

Ideally, the flow of nutrients should continue without interruption and at the same level as the fetus transitions to become an infant. Although the goal of an uninterrupted flow of nutrients is at present unattainable, it should be the goal that the caretaker strives to achieve. After all, it is not known what degree of shortfall from the goal, if any, is safe in terms of neurocognitive outcome.

Nutrients are initially delivered to most VLBW infants parenterally. While nutrients are provided parenterally, trophic feedings are initiated with the objective of fostering maturation of the immature intestinal tract. When maturation has advanced to the point where full feedings can be administered, parenteral nutrition can be terminated. This marks the beginning of the late feeding period, during which enteral feedings are the exclusive source of nutrients. Growth similar to the fetus, or faster than the fetus, is by consensus optimal. Yet, growth frequently proceeds at a slower rate (postnatal growth failure).

The reason for growth failure is mainly inadequate protein intake, which in turn is caused by the low protein content of most feedings. Inadequate protein intake is thus ultimately responsible for the poor neurocognitive development with which growth failure is associated [22]. Therefore, the achievement of adequate protein intake is mandatory if neurocognitive impairment is to be minimized.

45.7.1 Parenteral Nutrition

Delivery of nutrients via the parenteral route should commence immediately at birth. Nutrients may initially be limited to amino acids, energy in the form of glucose, and certain minerals, such as calcium, phosphorus and magnesium. Full parenteral nutrition, including intravenous lipids, should be instituted within 24 hrs of birth. Parenteral nutrition starting within 2 hours of birth has been shown to be effective and safe [23]. Delivery of amino acids in the needed amounts (Table 45.1) presents no difficulty, whereas delivery of energy can usually not be achieved for some days without incurring hyperglycemia, even if lipids are provided. The best strategy is to start glucose at a rate of 4.2 mg/kg/min and to increase delivery stepwise as long as euglycemia is maintained. Lipid emulsions based on soy oil provide needed energy and deliver modest amounts of the essential long-chain polyunsaturated fatty acids, docosahexaenoic acid and arachidonic acid. Parenteral nutrition should be continued until enteral feedings provide >90% of the required nutrients.

45.7.2 Early Enteral Nutrition

While adequate amounts of nutrients are being delivered via the parenteral route, enteral feedings should be started soon after birth. The sole objective of early (trophic) feeding is to stimulate the immature intestinal tract to undergo maturation. A marker of the intestinal tract's immaturity are the frequent gastric residuals, which can therefore be used to follow the intestinal tract's maturation. The best trophic feeding is human milk because of its superior ability to mature the gut while keeping the risk of necrotizing enterocolitis to a minimum. Because it usually takes some days for the mother's milk to become available, donor milk may be used to initiate gut stimulation in a timely manner, i.e., on the first day of life.

Gastric residuals should be monitored and their size taken into account in advancing feedings. It is probably prudent to keep the volume of trophic feedings low (<10 mL/kg/d) until gastric residuals are substantially diminished. There is lack of consensus on whether feedings should be kept low for a fixed period, e.g., six days, or whether cautious advancement can begin sooner. Contrary to earlier perceptions, neither the age at which trophic feedings are started, nor the rate of advancement of feedings have an impact on the risk of necrotizing enterocolitis [24]. Regardless of how feedings are advanced, it is important not to withhold feedings for more than a few hours because persistent feeding is crucial for optimal bowel maturation. Vigilance for signs of necrotizing enterocolitis must be maintained at all times. However, gastric residuals are not an early sign of necrotizing enterocolitis and their monitoring is not of proven value in the prevention of necrotizing enterocolitis. Delays in reaching full feedings should be minimized as early achievement of full feedings offers relative protection against late-onset sepsis [25].

45.7.3 Late Enteral Nutrition

It is widely appreciated that human milk does not provide the nutrients required by premature infants (Tables 45.1 and 45.3). The nutrient content of human milk must therefore be increased by the addition of nutrient supplements (fortifiers). Infants fed unsupplemented human milk show slow growth, which carries the risk of impaired neurocognitive development. In addition, premature infants can develop deficiency states of specific nutrients, such as osteopenia (Ca, P) or zinc deficiency. Commercially available human milk fortifiers provide protein, energy and the necessary minerals and vitamins. The amounts of minerals and vitamins are designed to meet or exceed, together with the nutrients intrinsically present in human milk, the concentrations specified in Table 45.4. Fortifiers provide energy in the form of carbohydrates and lipids because this enables the meeting of energy needs while keeping feeding volumes low. The protein provided by fortifiers (1.0–1.1 g/100 mL) is insufficient to meet the needs for protein at all times. Protein intakes approach protein needs if and when the protein content of human milk is at its highest, i.e., in the first two weeks of lactation. But it becomes progressively more inadequate as the protein content of human milk decreases with the duration of lactation. Inadequate protein intake from fortified human milk is the main cause of postnatal growth failure. Adequate protein intakes can be achieved if, in addition to the standard amount of fortifier, additional protein is added in a fixed amount, either as such or in the form of additional fortifier. Additional protein can also be added as part of a targeted fortification regimen [26] or in an adjustable fortification regimen [27]. There is no consensus regarding which of these methods is preferred. There is, however, no question that the amount of protein provided by commercial fortifiers is inadequate and that additional protein must be provided in some form.

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46

Enteral Feeding of the Very Low Birth Weight Infant

Johannes B. (Hans) van Goudoever

46.1 Introduction

Enteral nutrition is the natural way of feeding infants. The fetus receives enteral nutrition via the amniotic fluid. The amniotic fluid is largely composed of fetal urine, but lung fluids, nasopharyngeal secretions and intra- and trans-membranous fluids contribute as well. Amniotic fluid contains protein and carbohydrates. The amino acid concentrations of amniotic fluid resemble the fetal plasma amino acid concentrations. The fetus starts to swallow considerable amount of amniotic fluid in the last trimester. By term, the fetus swallows about 700 mL per day, corresponding with 170–230 mL/kg/d [1]. It is estimated that up to 10–15% of the nitrogenous requirement of the fetus can be met by swallowing amniotic fluid [2].

There have been concerns that preterm infants have limited capacity to process carbohydrates, fats and proteins. Peptidases, brush border enzymes and hydrolases are present in fetal life, with an increase in enzyme activity with the glucocorticoid surge just before birth [3]. Pancreas enzymes, such as trypsin, lipase and amylase are secreted into the duodenum by 31 weeks. From approximately 26 weeks of gestation, lingual and gastric lipases are present, which might help in digestion and absorption of lipids. Bile acids are already secreted by 22 weeks, although the synthesis is much lower in preterm compared to term infants.

The effect of extreme premature birth (<26 weeks) and antenatal steroids on digestion and absorption is not precisely known, but may enhance these processes. Lactase activity is also lower in preterm infants, but preterm infants display normal growth when they are fed lactose-containing milk. Preterm infants fed formula pretreated with lactase showed a modest increase in initial weight gain, an effect that disappeared at the end of the study [4]. Proteins are taken up rather efficiently in preterm infants, a process that might be facilitated by pinocytosis and also because of the increased intestinal permeability, demonstrated in several studies [5, 6], intact proteins such as lactoferrin can be absorbed [7]. Preterm infants fed hydrolyzed formula reached full enteral feedings approximately two days earlier than non-hydrolyzed formula [8], with similar protein metabolism indices [9]. Enteral nutrition itself may induce the maturation process of digestion and absorption and enhance tolerance to larger volumes of enteral nutrition [10, 11]. Altogether it follows that enteral nutrition should be installed as soon as possible, also to avoid the complications of parenteral nutrient administration. This chapter will focus on feeding mode and type of enteral nutrition.

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46.2 Feeding Mode

46.2.1 Minimal Enteral Feeding

The most important reason for withholding enteral feedings is necrotizing enterocolitis (NEC). This serious gastro-intestinal problem affects approximately 5% of very low-birth weight (VLBW) infants, but the incidence may vary widely amongst centers. Mortality rates are high, ranging from 20-40%. The multifactorial etiology includes gut hypoxia, impaired intestinal host defense mechanisms and alterations in microbial intestinal content (in amount, diversity and timing of colonization). Enteral nutrition is considered a risk factor as well, since in preterm infants, NEC develops in the second or third week of life, after the introduction of enteral feedings [12]. However, prevention of NEC by withholding enteral nutrition has not been shown. On the contrary, withholding enteral nutrition to neonatal animals reduces intestinal cell proliferation and gut mass [13]. In rats, lack of enteral nutrition results in atrophy of the proximal gut mucosa [14]. On the other hand, introducing large amounts of enteral feeding is a way of establishing NEC in premature piglets, despite the

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

fact that in utero large amount of amniotic fluids are ingested [15, personal communication]. Large amounts of enteral feedings are frequently not tolerated by the VLBW infants. Poor motility, pathogenic microorganisms, and inflammatory processes are related to the differences observed in postnatal life compared to prenatal life [12].

Many trials have examined the effects of minimal enteral feeding in VLBW infants [11, 16–20]. Many different definitions exist, but in general these are considered relatively small enteral volumes of human milk or formula (<25 mL/kg/d). They can be started as early as day one of life, even in extremely low birth weight infants. In neonatal piglets, mucosal growth is not observed before 30% of the total amount of nutrition is administered enterally, so minimal enteral feeding is not intended to serve as a nutritional source [13]. The initially observed physiological effects were increased motility and subsequent improved feeding tolerance resulting in higher weight gain rates, but recent results do not confirm this finding [19–21]. Gut hormone levels are stimulated by minimal enteral nutrition, possibly explaining the improved motility [16]. Also less need for phototherapy and reduced rates of osteopenia and cholestasis have been reported, probably all related to a reduced time on parenteral nutrition [10, 11, 17, 18]. Minimal enteral nutrition does not affect the incidence of NEC [21].

Lack of any enteral nutrition is associated with an increase in neonatal late onset infections [22]. This might be due to bacterial translocation from the intestine. In addition, the intestinal immune/inflammatory response is affected by the route of feeding, with an altered balance of cytokines, lower IL-4 and IL-10 levels in the non-enterally fed state and subsequent lower IgA production [23, 24]. Enteral nutrition has been demonstrated to down regulate the intestinal inflammatory response [25]. However, clinical trials have not demonstrated a beneficial effect of minimal enteral feeding on invasive infections [20].

Although large clinical trials on minimal enteral nutrition in VLBW infants are lacking, many studies including animal studies show beneficial effects of minimal enteral feeding in growth and feeding tolerance and provide indications of improved defense mechanisms against bacterial infections and inflammatory processes. This favors initiation of minimal enteral feeding shortly following birth as no adverse effects are reported.

46.2.2 Bolus or (Semi-) Continuous Feeding

Bolus feeding results in a significant increase in blood flow to the portal-drained viscera (i.e., stomach, spleen, intestine and pancreas). There is a surge in gastro-intestinal hormones, which is higher than when neonates are fed continuously [26]. Clinical trials show that even very small preterm infants tolerate bolus feeding well. Smaller infants seem to benefit more from continuous feeding [27, 28] and a recent small trial showed that preterm infants underwent more behavioral stress when fed intermittently [29]. However, a large randomized controlled trial with 250 VLBW infants has just been finished and the preliminary results show that even infants less than 1000 g tolerate bolus feeding well [W. Rövekamp-Abels, personal communication]. Some older trials have demonstrated less tolerance to intermittent bolus feeds than continuous milk infusion. Bolus feeding may result in apnea, deteriorating respiratory mechanics with decreased tidal volumes and transient hypoxia [30, 31]. These complications might aggravate in the presence of blocking materials of the nostrils such as nasogastric tubes, or CPAP devices. In conclusion, there is still not enough evidence to recommend either bolus or (semi-) continuous feeding as the preferred method.

46.2.3 Oral Feeding

Oral feeding is possible when an infant is capable of an adequate suck-swallow reflex. In the term infant the full, complex, integrated mechanism of swallowing, with the movement of the bolus of milk into the stomach, protection of the airway, inhibition of respiration and appropriate relaxation of the esophageal sphincter and gastric fundus is achieved within two days of birth [32]. The swallow function is present from 16 weeks of gestational age, and gastrointestinal motor activity, in small bursts, from 24 weeks onwards. Organized motility is present from around 30 weeks of gestation and nutritive and swallowing function from 32 weeks. Enteral nutrition promotes postnatal maturation of intestinal motor activity. It follows that oral feeding is only possible from 32 weeks onwards. Non-nutritive sucking can start earlier and may result in more rapid weight gain or earlier discharge although the results are not consistent [33]. The preterm infant of less than 32 weeks may be put on the breast, but the likelihood that the infant will ingest a significant volume of milk is very small. However, it may offer the mother a considerable psychological benefit and should therefore be strongly encouraged.

46.2.4 Intragastric Feeding

As oral feeding is not possible in VLBW infants, most frequently gastric tubes are used. Either nasogastric or orogastric tubes can be installed, both checked in the right position by checking the aspirate for acidity with litmus paper or by injection a small amount of air while listening with a stethoscope placed over the stomach. The nasogastric tube has the advantage of easier fixation but has the disadvantage of (partially) blocking one nostril. With the use of some CPAP devices, a nasogastric tube is not possible. With the use of tubes and syringes, one should be aware of a potential loss of nutrients such as lipids and calcium that may not reach the infant [34].

46.2.5 Transpyloric Feeding

Although transpyloric feeding is frequently used in pediatric intensive care [35–37], it should not be used in neonatal intensive care. Mortality rates are increased in infants that received transpyloric feeding, although this study might have been affected by selective allocation of the less mature and sicker infants to transpyloric feeding. No proven benefits in tolerance, growth or in aspiration rates have been reported consistently [33, 38].

46.3 Type of Enteral Nutrition

The aim of the nutritional support of the low birth-weight infant is to mimic intrauterine growth rate and obtain a long-term functional development similar to the term infant. The nutrient requirements for the VLBW infant are described elsewhere (see Chapter 45), but in general nutritional support should be designed to compensate for metabolic and gastrointestinal immaturity, immunological compromise and other problems associated with prematurity.

Own mother's human milk is the preferred choice, even for preterm infants although it does not provide the required nutrients. Fortification is thus necessary, as unfortified human milk leads to suboptimal growth [39–41]. Human milk is superior to cow's milk based formulas because of the species specificity. This results in the availability of, for instance, proteins and oligosaccharides important for the host defense, which might explain the reduced rates of NEC observed in human milk fed infants [42, 43]. Interestingly, pasteurized donor milk does not seem to reduce the incidence of NEC, although for instance oligosaccharides are not influenced by pasteurization [44, 45]. Bio-availability of calcium and phosphorus is higher in human milk, but preterm infants still frequently need supplementation. Lipases are present in breast milk, which might enhance fat absorption. Bacterial flora in infants fed preterm formula differs markedly from those who have been fed human milk, although the recent improvements in preterm formula facilitate the growth of *Bifidobacterium* and *Lactobacillus* species as well [46–48]. Human milk is better tolerated than formula, with a faster gastric emptying [49], while human milk might reduce intestinal permeability as well [50]. Long-term benefits of human milk include lower blood pressure, lower LDL to HDL ratio and possibly a reduced risk of obesity and higher IQ [51–53]. Early feeding with a higher nutrient dense formula results in a similar IQ to that found in breastfed children [54].

46.3.1 Preterm Human Milk

The milk from mothers, who have delivered prematurely, so-called preterm human milk, varies significantly from milk from mothers who delivered at term. Concentrations of proteins, lipids (and thus energy), vitamins, calcium, sodium and trace elements are higher [1, 2]. However, the concentrations of these nutrients soon decline and do not meet the requirements after approximately two weeks. In addition, clinical problems such as patent ductus arteriosus and bronchopulmonary dysplasia often restrict fluid intake demand fluid restrictions with the consequent need for nutrient dense feedings. The adequacy of nutrient intake is further compromised by the variability in composition. For example, the within-feed change in lipid content is well known and can be 2-3 folds different between fore- and hind milk.

46.3.2 Expressed Mother's Milk

As most low birth weight infants are not able to drink from the breast directly, most mothers choose to express their milk and deliver the milk to the unit themselves. As human milk is not homogenized, the fat content may separate from the remaining milk. Fresh milk is favorable, since freezing and thawing reduces the function of some beneficial components in human milk. Light exposure of breast milk reduces the riboflavin and vitamin A content substantially within a few hours. Human milk, even when fortified, can be stored at refrigerator temperature for 72 hours [34].

46.3.3 Human Milk Banks

An alternative to own mother's milk is the use of milk from a milk bank. Setting up a milk bank may be costly and after setting up the average cost per liter is around €30/liter [55]. Many units around the world have such a milk bank, where mothers donate their milk to be used, most often without payment. Although some argue that banked milk does not need to be pasteurized, almost all banks do, due to fear of pathogens. Human milk can transmit infection. Donors should be screened for hepatitis B, hepatitis C and HIV, with counseling offered when needed. Pasteurization might destroy HIV [56]. HIV is the most well recognized viral pathogen, but transmission of cytomegalovirus is not uncommon, and raw breast milk can cause serious and even fatal illness in preterm babies [57]. Pasteurization, freezing and thawing lower antimicrobial factors and denature milk lipase [58]. It may reduce vitamin content as well. Therefore, benefits are not as clear as with non-pasteurized, own mother's milk. On the other hand, most of the lysozyme, IgG and almost all IgA remain intact. A meta-analysis of trials in preterm newborns, all conducted in the early 1980s, showed that exclusively donor breast milk reduced the risk of NEC—a serious inflammatory condition of the bowel—when compared with formula [59]. Donors have usually delivered at term or have been lactating for some time, both of which re-

sult in lower nutritional content. Nowadays commercial instruments are available that can screen the nitrogen and energy content of milk very easy, to be informed about some quality aspects of the donor milk.

46.3.4 Fortifier

Exclusively unfortified, human milk fed preterm infants show lower serum albumin, total protein and transthyretin concentrations as a result of an inadequate protein intake. Growth rates are lower than fetal growth rates [60, 61]. In addition, calcium and phosphorus content are too low to meet the preterm infants requirements, resulting in decreases in serum phosphorus and increases in alkaline phosphatase [62] in the short-term and linear growth reduction in the long term [63]. Hyponatremia may result in a later stage, especially when diuretics are used.

The protein related deficits that might occur using unfortified milk can be corrected with supplementation [60, 61, 64]. Protein fortification has been shown to increase weight gain [65, 66]. Both human milk protein sources as well as bovine protein supplementation has been used, with similar results [67]. A recent study showed that human milk based fortifier resulted in a lower NEC incidence than cow milk based fortifier and preterm formula although the NEC incidence in the formula group was very high (16%).

Supplementation with calcium and phosphorus is possible as well. Indices of bone health, such as urinary calcium and phosphorus excretion, normalize with supplementation. Length gain improves, as well as bone mineralization in the long term [68].

The vitamin K concentration of human milk is very low and for the newborn breastfed infant, a deficiency state has been described. Vitamin K is produced by bacteroides and *E. Coli*, bacteria that are usually not common in breastfed infants. However, if prophylactic vitamin K is given at birth, no signs of deficiency have been described for three months [69], although the addition of vitamin K in fortifiers is logical and current practice.

As human milk composition changes in time, even fortified milk might not meet the requirements of the rapidly growing preterm infant. Frequent monitoring of the administered amount of nutrients remains necessary. Milk analyzers should therefore be available on all units as standard of care. The policy of waiting to change the composition of the nutrient intake until growth is faltering should be considered as suboptimal therapy, which can seriously affect long-term outcome.

Human milk fortifiers have been criticized for possible side effects, such as a loss of intrinsic host defense. As described above, there is an indication that infants fed a human milk based fortifier with human milk might be less prone to develop NEC. In a study, comparing either preterm formula as supplement or bovine fortified pasteurized human donor milk, no differences in sepsis or NEC were noted. Both groups showed a higher incidence when compared to human milk fed infants fortified with human milk fortifier [44]. No effect of fortifier is noted on IgA concentration [70, 71]. Another fear of the use of fortifiers is a reduced tolerance. A recent meta-analysis has revealed no differences between infants fed fortified or unfortified human milk [68]. Also no differences were observed between infants fed preterm formula or fortified human milk [72].

46.3.5 Preterm Formula

Although the goal of providing nutrients to the preterm infant is to achieve a functional outcome similar to that of the breastfed term infants, it is obvious that the composition of preterm formula is different from term formula, since the requirements are much higher. Until now, the factorial approach has been used, with only moderate success. With the factorial approach, estimations of requirements are made based upon intrauterine accretion rates, while accounting for endogenous losses, incomplete digestion and absorption. Intrauterine accretion rates are obtained from carcass analyses of fetuses, with sometimes limited information of the nutritional status of the mother or possible diseases and on intrauterine growth curves. Subsequent modifications of such formulas were made following clinical studies. As stated above, this approach has yielded only limited success. Weight gain rates are lacking behind intrauterine rates, especially in the extremely low birth weight infants [73, 74]. Mainly retrospective studies have shown detrimental effects of weight gain rates that are suboptimal [75, 76]. Very recently, guidelines developed from the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) together with a group of experts have indicated major changes in requirements, which are discussed elsewhere (see Chapter 45). Readers are referred to these published recommendations for a detailed description.

46.4 Approach to Improve Enteral Feeding

As yet, current enteral feeding strategies fail to meet the requirements of VLBW infants. Many more clinical trials are needed to improve the quality of the nutrient supply. An alternative approach would be to determine the quantity and quality of intrauterine nutrition by measuring fetal intake and fetal metabolism. Obviously, there are many differences between life in or outside the uterus. Compared to the fetus, preterm infants are depending on their own metabolism, use their lungs, and are subject to more gravity forces. In addition they are frequently ill, with might demand specific nutrients to support defense mechanisms. Medication usage may alter

metabolism. Their intestines start to be colonized, and bacteria have their own nutrient requirements. However, intrauterine nutrition and metabolism might provide very valuable insight in to what the fetus is capable of with regard to metabolic activity, although the mother plays a role in the conversion to and excretion of metabolic end products.

Glucose is the major oxidative substrate in utero. The ovine fetus utilizes glucose at a rate of approximately 4-7 mg/kg/min, but has the capability to metabolize double [77]. Lactate is also important as energy source, especially when other sources of energy are lacking [78]. When born at term, the human baby has a large portion of fat. Fat deposition increases exponentially with gestational age. Near term the fat accretion is 7 g/day. In the fetal sheep, large quantities of amino acids are taken up by both the placenta and the fetus [79]. About half of the retained amount is used as fuel source and the ammonia produced in this process is converted into urea by the fetal liver. Recent studies show a very active amino acid transport in the human fetus as well [80]. Comparison of albumin synthesis rates, using stable isotope techniques, revealed that very immature fetuses produce large quantities of albumin, rates that are not reached following birth with the present nutritional strategies [81]. This information provides guidelines as to which extent anabolism can occur and to which extent postnatal nutrition should be provided.

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47

Parenteral Nutrition

Jacques Rigo and Thibault Senterre

47.1 Introduction

Modern perinatal medicine has resulted in dramatic decrease of mortality in premature infants, especially very low birth weight (VLBW, <1500 g) infants. With the major advances in life-support measures, nutrition has become one the most debated issues in the care of low-birth-weight infants. In this regard, several reports have shown the major effect of quantitative and qualitative nutrition during the first period of life on early and late outcome.

Incidence of intrauterine growth restriction (IUGR) is relatively high in VLBW infants and postnatal growth restriction (PNGR) is frequently observed during the early weeks of life resulting in a major growth deficit at the time of discharge. According to population, clinical disorders and nutritional support, growth restriction affected 60–100% of VLBW and extremely low birth weight (ELBW) infants [1].

PNGR is mainly the result of the cumulative nutritional deficit occurring during the transitional period from birth to the time of full enteral feeding [2, 3] but additional effects during the stable growing period have also been suggested [4]. Therefore, several studies evaluated the effect of more optimal (aggressive) nutrition during the early weeks of life [5–8] and various international scientific committees reconsidered current nutritional recommendations in preterm infants focusing on ELBW and VLBW infants (Table 47.1) [9, 10].

Due to gastrointestinal tract immaturity, cardio-respiratory adaptation, clinical status and high nutritional requirements, VLBW infants require parenteral nutrition from the first days of life to promote early positive nitrogen retention and growth. In addition, parenteral nutrition could be required or prolonged during the stable "growing" period due to feeding intolerance, gastrointestinal disorders or surgery. Provision of parenteral nutrition (PN), total or suppletive, can be ordered using individualized prescription, homemade standardized parenteral solution or ready to use industrialized prepared multichamber bags.

In this chapter we discuss the most important features regarding parenteral nutrition that have been recently reviewed [11–14] (Table 47.1), outlining more recent practical aspects and guidelines particularly for ELBW and VLBW infants.

47.2 Nutritional Support in VLBW Infants

Nutrition of VLBW infants may be divided into two subsequent periods, firstly the immediate adaptive or "transitional" period after birth, and a stable "growing" period up to discharge from the NICU. Depending on birth weight (BW) and gestational age (GA) the transitional period may be prolonged, particularly in the more vulnerable infants with major clinical disorders. The more premature a neonate, the more challenging are the influences of immaturity and the accompanying morbidity on nutritional supply. Most of these infants receive parenterally delivered nutrients as their major source of nutrition for the first days, and sometimes weeks, of life.

Nutrition during the "stable-growing" period on PN is exceptional, used only when preterm infants are recovering from surgery and/or severe gastrointestinal problems, to prevent or limit the use of the gastrointestinal tract.

47.2.1 Parenteral Nutrition During the Transitional Period

The aim of the nutritional support in VLBW infants during the transitional period is:

1. to reduce protein catabolism providing an energy intake at least at the level of energy expenditure;
2. to provide amino acids (AA) at a level sufficient to induce a positive nitrogen retention;

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Table 47.1 Recent recommendations of daily parenteral intakes for ELBW and VLBW infants

		Tsang &all 2005 [9]			ESPEN-ESPGHAN [12]		
		Day 0	Transition	Growing	Day 0	Transition	Growing
Fluids	mL/kg/day	90–120	90–140	120–180	80–90	100–150	140–180
Energy	kcal/kg/day	40–50	75–85	90–115	50–60	60–100	100–120
Protein	g/kg/day	2	3.5	3.5–4	1.5–2.5	2.0–3.5	3.5–4
Carbohydrate	g/kg/day	7	8–15	13–17	7–10	9–15	12–18
Fat	g/kg/day	1	1–3	3–4	0–1	1–3	2–4
Sodium	mmol/kg/day	0–1	2–5	3–7	0–3	2–3	2–7
Potassium	mmol/kg/day	0	0–2	2–3	0–2	1–2	2–5
Chloride	mmol/kg/day	0–1	2–5	3–7	0–3	2–3	2–5
Calcium	mmol/kg/day	0.5–1.5	1.5	1.3–4	NP	1–2	1.3–3.0
Phosphorus	mmol/kg/day	0–1	1.5–2	1.5–2	NP	1–2	1.0–2.5
Magnesium	mmol/kg/day	0	0–0.3	0.2–0.3	NP	NP	0.2–0.4
Zinc	µmol/kg/day	0–2	2,5	6	NP	3	4

NP not provided.

3. to provide water and electrolytes to control hydration status and postnatal physiological fluid adaptation;
4. to progressively increase AA, energy and mineral intake to promote nitrogen retention and early growth and to limit a possible cumulative nutritional deficit;
5. to limit postnatal metabolic stress in this high-risk population.

Recent data suggest that provision of a more "aggressive" nutritional support from the first day of life reduces postnatal growth deficit and improves neurodevelopmental outcomes in VLBW infants [15, 18].

47.2.1.1 Fluids and Electrolytes

A number of adaptive processes occurring at birth affect nutritional support. During the early weeks of life, water, electrolyte and mineral homeostasis, and glucose control are more challenging. Placental clearance and placental supply of fluids, electrolytes, minerals and nutrients are discontinued. Thermoregulation and insensible water losses influence water metabolism, especially in VLBW infants. Subsequent adaptation and compensatory regulative processes need time to stabilize.

Fluid and electrolyte administration during the transitional period should allow contraction of extracellular fluid space without compromising intravascular fluid volume and cardiovascular function, maintain normal plasma electrolyte concentrations, without impairing urinary output (<0.5–1.0 mL/kg/h) for more than 12 hours.

Water balance may be very unstable, especially in ELBW infants, urinary output may be high (6–10 mL/kg/h) and insensible fluid losses can be huge, especially under a radiant warmer and/or with phototherapy.

A negative net balance for sodium is allowed and intakes should be less than 2 mmol/kg initially. Restricted sodium intake in VLBW infants has a positive influence on oxygen supply and risk of later bronchopulmonary dysplasia. However, high sodium urinary excretion (10 mmol/kg) may occur, especially in case of high fluid perfusion over 170–200 mL/kg/d compromising sodium balance.

This transitional phase may last for a variable period of several hours or days and result in initial weight loss. The end of the transitional period is usually characterized by a urine volume <2.0 mL/kg/h, urine osmolarity>serum osmolarity, fractional Na excretion diminishing from >3% to <1% and specific gravity above 1012. In preterm infants, these changes complete after 3–5 days and water and electrolytes intake will be progressively increased to replete the body losses.

During the transitional period, VLBW infants should be weighted twice daily and in/out balance, plasma sodium concentration and urinary excretion should be monitored attentively during first days of life. Fluid intake on PN should be 50–100 mL/kg/d in VLBW infants according to clinical and environmental conditions and sodium and potassium intake limited to less than 2 mmol/kg/d [12, 14].

47.2.1.2 Amino Acids

To reduce the temporary interruption of the transfer of nutrients, to limit the high protein catabolism up to 1.5 g/kg/d and to induce a positive nitrogen balance from the first day of life a high-protein supply (>2 g amino acids/kg per day) has recently been suggested in the so-called "aggressive" nutrition [6–8].

Although, long-term benefits were not clearly demonstrated, it has been suggested that high protein intake during the first week of life induces positive nitrogen balance, increases insulin secretion, improves glucose tolerance, promotes early weight gain and improves neurodevelopmental outcome at 18 months [17]. Thus, new recommendations [12, 14] suggest providing 2–3 g of AA on the first day of life using a parenteral solution with a high AA:energy ratio and to progressively increase the AA intake up to 4 g/kg/d at the end of the first week of life.

47.2.1.3 Energy

Energy intake is required for both protein metabolism and deposition. Theoretically, an energy intake approximating the resting energy expenditure (i.e., 40–60 kcal/kg/day) allows the minimization of protein catabolism to about 1.5 g of protein/kg/d in ELBW infants. If amino acid intake is adequate and energy intake is in excess of resting energy expenditure, weight gain is achieved. However, because of individual differences in energy expenditure, the resting energy requirement varies considerably in this population. New recommendations [12, 14] suggest providing 40 kcal/kg/d on the first day of life, to increase up to 75–85 kcal/kg/d during the transitional period and to reach close to 100 kcal/kg/d at the end of the first week of life.

47.2.1.4 Carbohydrates

Glucose homeostasis is still immature during the early days of life in VLBW infants who are subject to hyper or hypoglycemia. Glucose is the main carbohydrate in fetal life and approximately 7 g/kg/d (4 mg/kg/min) of glucose crosses the placenta in the last trimester of pregnancy. Glucose production around 8 mg/kg/min (11.5 g/kg/d) in preterm infants is maximal in the postnatal period and decreases gradually with age. Gluconeogenesis may be responsible for a part of glucose production. During high rates of glucose infusion, endogenous production is not completely suppressed in VLBW infants. By contrast, maximum glucose oxidation is relatively limited, 7–8.5 mg/kg/min (10–12 g/kg/d), but could be less in critically ill VLBW infants. The imbalance between glucose infusion rate and endogenous production on one hand and the maximum oxidation rate explain the increasing incidence of hyperglycemia in VLBW infants. In addition, due to their immaturity and their underlying diseases, VLBW infants are relatively resistant to insulin during the first week of life.

During the transitional phase, fluctuations in blood sugar levels are frequently observed resulting from an insufficient glucose and energy intake associated with low substrate reserves (hypoglycemia) or from a relative excess of glucose and energy intake associated with some degree of insulin resistance (hyperglycemia). Although the definition and the long-term consequences of neonatal hypo- and hyperglycemia remain controversial, plasma glucose concentration should be monitored to remain in a normal range for VLBW infants on parenteral nutrition between 50 mg/dL (2.75 mmol/L) and 150 mg/dL (8.3 mmol/L). Hyperglycemia can be decreased by reducing insensible water loss, glucose infusion rate and by providing exogenous insulin supply. Insulin administration may help to control plasma glucose concentration, to achieve increased energy intake and to promote nitrogen retention and growth, although there is need for more data on its safety and long-term consequences as a growth-promoting agent. More recently, it has been proposed that high AA intake from the first day (2–3 g/kg/d) improves glucose tolerance in ELBW infants by stimulating growth, by enhancing insulin and insulin-like growth factors secretion [6]. This approach requires further randomized control trials.

In practice, 6 g glucose/kg per day are generally well tolerated (4–5 mg/kg/min) even on the first day of life in VLBW infants. If this intake is tolerated, it may be increased progressively to 12–16 g/kg per day at the end of the first week of life. If it is not tolerated, progression of glucose intake will be reduced and insulin perfusion will be considered according to clinical and nutritional status with an initial dose of 0.05 IU/kg/h.

47.2.1.5 Lipids

Intravenous lipid emulsions are important constituents of total PN as they provide in an isotonic solution, high energy density and essential fatty acids to VLBW infants. Intravenous lipids play two separate roles in the PN of VLBW infants. The first role is as a high-density energy substrate to be readily utilized by VLBW infants. The other role is as a source of essential fatty acids as well as long-chain PUFAs. Essential fatty acid deficiency is avoided by infusions of 0.5–1.0 g lipid per kg per day. The importance of long-chain PUFAs for the development of the brain and the retina has also been recognized. Intravenous lipid emulsions contain small amounts of these fatty acids as part of the egg phospholipid used as a stabilizer. However, clearance of lipid emulsion could be impaired in ELBW infants particularly those with IUGR requiring the monitoring of triglyceridemia.

Actually, new recommendations [12, 14] suggest providing 1 g/kg/d on the first day increasing stepwise fashion to 3.0 g/kg/d at the end of the first week of age.

47.2.1.6 Minerals: Ca, P and Mg

Calcium and phosphorus transfer and retention is high during the last trimester of gestation. Combined with a relative immaturity of hormonal control (Vit D, PTH), VLBW infants particularly are at risk of early neonatal hypocalcemia and hypophosphoremia (see Chapter 49).

Calcium supply needs to be provided from the first day of life in combination with an adequate calcium phosphorus ratio to limit the risk of hypocalcemia and/or hypophosphoremia. Phosphorus plays a critical role in energy metabolism, and deficiency of phosphorus results in clinical disease, including muscle weakness. Early phosphorus deficiency is also potentiated by IUGR. Reference values for plasma phosphorus concentration differ in adults (>1.0 mmol/L, 3 mg/dL) and in preterm infants (>1.6 mmol/L, 5 mg/dL). Unfortunately, most neonatologists are unaware that the laboratory reports plasma phosphorus concentration of VLBW infants with regard to adult references and tolerates hypophosphatemia with

the risk of hypercalciuria and osteopenia. Optimal calcium to phosphorus ratio differs in parenteral and oral nutrition due to the bypass of the gastrointestinal tract; phosphorus retention is related to bone mineralization with a weight-to-weight calcium:phosphorus ratio of 2.15:1 but also to nitrogen retention with a weight-to-weight nitrogen:phosphorus ratio of 15:1. Therefore optimal Ca to P ratio in parenteral nutrition ranges between 1.5 and 1.3.

Magnesium is rarely adjusted even in the transitional period unless the infant has persistent hypocalcemia secondary to hypomagnesemia, or has abnormally high magnesium levels due to maternal levels. Serum magnesium levels should be checked in any small infant whose mother was treated for hypertension or preeclampsia.

Actually, new recommendations [12, 14] suggest providing around 25–40 mg (0.6–1 mmol) of Ca/kg/d, 18–31 mg (0.6–1 mmol) of P/kg/d and 2.5–4.0 mg (0.1–0.2 mmol)/kg/d of magnesium on the first day and to progressively increase the intake according to energy and AA supplies up to 65–100 mg (1.6–2.5 mmol) of Ca/kg/d, 50–78 mg (1.6–2.5 mmol) of Phosphorus and 7–10 mg (0.3–0.4 mmol/kg/d) of magnesium.

47.2.2 Parenteral Nutrition During the Stable Growing Period

The aim of the nutritional support in VLBW infants during the stable growing period is:

1. to induce growth rate and protein accretion in the range of the fetal weight gain considering the lean body mass gain as reference;
2. to abolish the development of a cumulative nutritional deficit during the first weeks of life;
3. to reach, at the time of discharge and/or of theoretical term, an anthropometric parameter in the range of reference values for term infants.

47.2.2.1 Fluids and Electrolytes

Intravenous fluid is the carrying vehicle for parenteral nutrition. A fluid intake about 140–160 mL/kg/d in both VLBW and VLBW preterm infants allows for covering the water requirement for replacing water loss and providing enough extra water to build new tissues during the stable growing period. Fat mass is relatively free of water content. By contrast, lean body mass content is about 80%. Thus a weight gain of 20 g/kg/d containing 40% of fat results in a net storage of 13 g of water and 1–1.5 mmol of Na^+/kg/d.

Sodium and potassium requirements are in the range of 3–7 mmol/kg/d for Na^+ and 2–5 mmol/kg/d for K^+ in VLBW infants respectively. A mean intake of 3 mmol/kg/day of sodium and 2 mmol/kg/day of potassium seem to be appropriate to maintain normal plasma concentration for most stable, growing infants. In parenteral solution, sodium is frequently provided with phosphorus in the form of sodium glycerophosphate and limitation of sodium content also limits the phosphorus content. Chloride supply requires particular attention in parenteral nutrition. Chloride requirement is generally considered similar to sodium requirement. Chloride content in parenteral solution is related to several potential components, AA solution, sodium chloride, potassium chloride or calcium chloride and is difficult to control. However, chloride intake plays a role in the acid-base homeostasis and imbalance between $Na^+ + K^+$ and Cl^- promotes metabolic acidosis or alkalosis [19]. Therefore, monitoring of plasma and urinary electrolyte concentrations and appropriate correction remains recommended, during parenteral nutrition.

47.2.2.2 Amino Acids

Nitrogen requirement in parenteral nutrition is close to 95% of the enteral requirement, but corresponds in terms of g AA/kg/g to the figure in g protein/kg/d recommended in enteral nutrition, due to a relatively lower nitrogen content. Protein requirement has been recently reviewed according to fetal nitrogen accretion, lean body mass gain and the need to compensate early cumulative protein deficits during the transitional period. 3.5–4.5 g of AA /kg/d is recommended during the stable growing period in ELBW and VLBW infants [9, 10, 20].

47.2.2.3 Energy

In contrast to what is generally suggested, the energy requirement in PN approximates to that of enteral nutrition. In fact, the gross energy content, measured by bomb calorimetry, of 1 g of amino acid is lower than that of 1 g of protein. Similarly, gross energy content of glucose is less than that of more complex carbohydrates. In contrast, while in parenteral nutrition the metabolizable energy of amino acid and fat solutions are identical to the gross energy, the metabolizable energy of dietary protein and fat in oral nutrition represents about 90% and 80% respectively [21]. Consequently, the recommendation for energy intake during the stable-growing period in VLBW infants on parenteral nutrition is relatively similar to that in oral nutrition and corresponds to 110–130 kcal/kg per day in VLBW infants.

47.2.2.4 Carbohydrates

Glucose contributes to most of the osmolality of PN solution (510 mOsm/L for a 10% solution) by contrast to lipid solutions. An excessive glucose intake increases CO_2 production and may be responsible for hyperglycemia, cause lipogenesis, steatosis and may contribute to liver dysfunction. The maximum glucose intake should not exceed the glucose oxidation rate in

parenteral nutrition of 13–18 g/kg/d and more than 60–75% of the non-protein energy during the stable growing period.

47.2.2.5 Lipids

Intravenous lipid emulsions are important constituents of total parenteral nutrition as they provide most of the energy intake and essential fatty acids (EFA). The CO_2 production is lowered compared to PN with a high proportion of carbohydrates and the nitrogen metabolism can be improved by adding lipid emulsions to PN. Lipid oxidation depends on the overall energy intake and consumption, intake of carbohydrates and triglycerides and the carbohydrate/lipid ratio. Lipid oxidation decreases as the carbohydrate intake increases and is replaced by lipid storage.

Maximum fat oxidation occur when lipid emulsions provide 40% of the non-protein energy in newborns so it is recommended that lipid intake should provide 25–40% of non-protein energy with a maximum of 3–4 g/kg/day [12]. An increase in the concentration of plasma triglycerides is to be expected if the infusion speed of the lipid emulsion exceeds the speed of triglyceride hydrolysis that depends on lipoprotein lipase activity. In all cases, the triglyceride infusion dose should be adjusted to maintain a serum triglyceride concentration not exceeding 200–250 mg/dL, especially in ELBW or severely ill infants who may have limited lipid tolerance.

Concerns had been raised on the potentially adverse effects of lipid infusion on hemodynamics, infections or hyperbilirubinemia. It appears prudent to avoid high lipid supplies in infants with sepsis, impaired oxygenation, or severe hyperbilirubinemia and lipid emulsion infusion should be continued at least at 0.5–1.0 g/kg/day, which is sufficient to prevent essential fatty acid deficiency.

Lipid supply may result in enhanced lipid peroxidation and the formation of free radicals. An increased lipid utilization by reducing the carbohydrate/lipid ratio results in a reduction of lipid peroxidation and free radical formation. PN should be supplemented with multi-vitamin preparations including both vitamin C and vitamin E (alpha-tocopherol), which have anti-oxidative effects. Excessive exposure of the bottle to light should be avoided.

Carnitine is necessary for the transportation of long-chain fatty acids via the mitochondrial membrane and its oxidative metabolism. Because carnitine synthesis and storage are not sufficiently developed at birth, particularly in preterm infants, and because no commercial intravenous solution has carnitine, parenterally fed infants present low plasma and tissue carnitine levels that decline with postnatal age [22]. Although a meta analysis (based on 14 randomised, controlled studies) showed there to be no effect of carnitine supplementation on the metabolism of lipids, lipogenesis or weight gain [23], a carnitine supplementation of 15 μmol/100 kcal could be advisible for infants on total parenteral nutrition for more than 4 weeks.

47.2.2.6 Minerals: Ca, P and Mg

Calcium and phosphorus cannot be provided through the same parenteral solution at concentrations needed to support in utero accretion, because of solubility. With a fluid intake range of 120–150 mL/kg/day, it is advisable to supply 65–100 mg (1.6–2.5 mmol) of Ca /kg/d, 50–78 mg (1.6–2.5 mmol) of iP and 7–10 mg (0.3–0.4 mmol/kg/d) of Mg, corresponding to a Ca/P ratio of 1.3:1 by weight and 1:1 by molar ratio in the TPN solution. It must be underlined that this quantity of calcium provided by parenteral route is about 55–80% of that deposited by the fetus during the last trimester of gestation (120 mg/kg/day) but similar or higher than that obtained in enteral nutrition with the available preterm formula (see Chapter 49).

47.2.2.7 Trace Elements and Vitamins

Vitamin mixtures for parenteral use have been available since the early time of parenteral nutrition and the amounts provided were (and are) determined to a large extent by the preparations available. Today, additives of all trace minerals for which a deficiency has been demonstrated are available. However, little definitive information is available concerning the parenteral requirements of either trace minerals or vitamins in VLBW infants. Research concerning the parenteral requirements of these nutrients by infants, of course, is hindered by the difficulties both of measuring plasma concentrations of the nutrients using small volumes of plasma and of interpreting the physiological significance of plasma concentrations.

47.3 Practical Aspects of Parenteral Nutrition in VLBW Infants

47.3.1 Basic Components Available for Parenteral Nutrition in Preterm Infant

47.3.1.1 AA Solutions

Considerable improvement of parenteral amino acid solutions have occurred from the late 1960s when the source of intravenous protein was casein hydrolysate. More specific pediatric amino acid solutions have been designed in the early 1990s with high essential/non-essential AA ratios and conditionally essential amino acid content for use in preterm infants. At least three different "gold standards" have been proposed for premature infants: (1) the amino acid concentrations from the umbilical cord obtained following fetal cord puncture or after birth, (2) the amino acid concentrations of rapidly growing preterm infants receiving their mother's milk or human milk supplemented with human milk proteins, (3) the amino acid

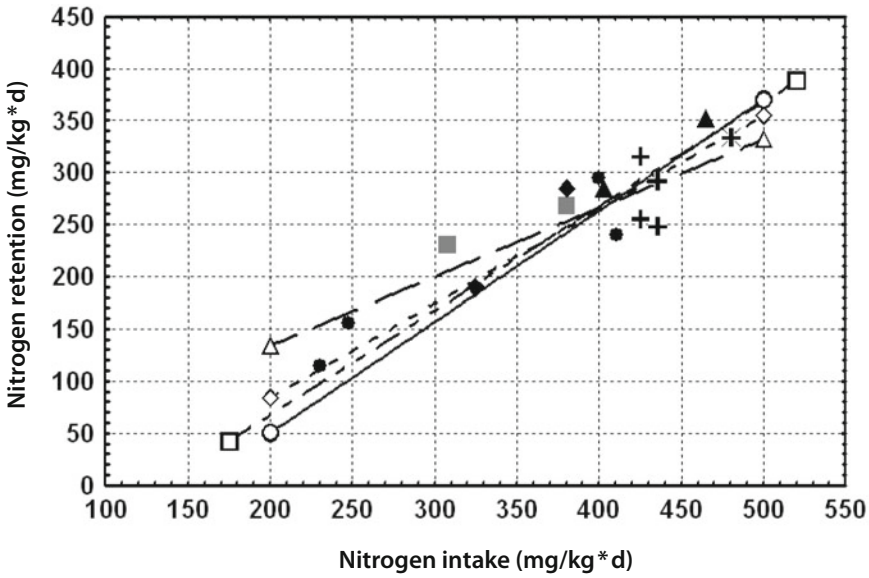

Fig. 47.1 Relationship between nitrogen retention and nitrogen intake in parenterally fed preterm infants computing of various studies (the different symbols represent individual studies)

concentrations of healthy breast-fed term infants. Nevertheless, despite the diverse composition of parenteral amino acid solutions used in pediatric care, nitrogen utilization does not change significantly (Fig. 47.1) [11, 20] (Table 47.2).

Therefore, optimal amino acid patterns for parenteral amino acid solutions in preterm infants still need to be determined and new solutions should be developed to potentially improve sulfur and aromatic AA imbalances and to provide additional glutamine. However, up to now, the use of cystein-HCl, acetyl-cystein, acetyl-tyrosine [24] and the supplementation with glutamine [25] have not demonstrated beneficial effects. The recent data evaluating the effect of bypassing the intestine on individual AA requirements has not been translated to designing and evaluating new AA solutions.

47.3.1.2 Lipid Emulsions

Intravenous lipid emulsions consist of different oils (soybean, safflower, coconut, olive and fish oils), egg yolk phospholipids and glycerol [reviewed in 12, 14, 26, 27] (see Table 47.3). Intravenous lipid emulsions provide high caloric, isotonic solutions and can also be given through peripheral lines. Traditionally, lipid infusions are prepared from soybean oil triglycerides emulsified with egg yolk phospholipids. Typical soybean oil contains about 45–55% linoleic acid (18:2n-6) and 6–9% linolenic acid (18:3n-3), but very little saturated or monounsaturated fat. Although clinically safe, experimental reports indicated that soybean oil based lipid emulsion could exert a negative influence on immunological functions. Those findings were related to its absolute and relative excess of ω-6 polyunsaturated fatty acids (PUFA) and the low amount of ω-3 PUFA and also to its high PUFA content with an increased peroxidation risk. The new lipid emulsion was basically designed in order to obtain balanced levels in polyunsaturated (ω-6 and ω-3), monounsaturated, and saturated fatty acids. They are differentiated by their fatty acid content, as well as fatty acid source of their origin, including soy, safflower, coconut, olive, and fish oil. Newer emulsions comprise physical mixtures of either a 20:80 mixture of soy ω-6 PUFA and ω-9 medium-chain monounsaturates (MUFA) from olive oil or a 1:1 ratio of LCT with coconut oil-derived medium chain triglycerides (MCT). Structured MCT/LCT emulsions formulated from a random combination of triglycerides synthesized on the same glycerol carbon chain are cleared faster from blood in moderately catabolic patients. The newer lipid com-

Table 47.2 Main composition of commercial parenteral amino acid solutions for preterm infants

Product	%	Total AA (g/L)	EAA (%)	Cyst(e)ine (g/L)	Tyrosine (g/L)	Taurine (g/L)	Osmolality (mosm/L)	pH
Aminopäd	10	100	42	0.5*	1.1***	0.3	790	6.1
Aminoplasmal	10	100	42	**	0.4	–	864	5.7-6.3
Primene	10	100	48	1.9	0.5	0.6	780	5.5
Aminoven infant	10	100	51	0.5	4.2***	0.4	885	5.5-6.0
Vaminolact	6.5	65	44	1.0	0.5	0.3	510	5.2
TrophAmin	10	100	49	**	0.2	0.3	875	5.5

EAA Essential amino acid (n = 8), * As acetyl-cystein, ** Separately as cystein HCl, *** As acetyl-tyrosine.

Table 47.3 Oil content (%) in commercially manufactured intravenous lipid emulsions

Product	Soy (LCT)	Coconut (MCT)	Olive (MUFA)	Fish (ω3)
Intralipid	100	0	0	0
Lipofundin MCT/LCT	50	50	0	0
Structolipid	64	36	0	0
ClinOleic	20	0	80	0
LipoPlus	40	50	0	10
SMOFlipid	30	30	25	15
Omegaven	0	0	0	100

LCT long-chain triglycerides, *MCT* medium-chain triglycerides, *MUFA* mono unsaturated fatty acid.

binations with a smaller proportion of soy oil, have a much lower content of linoleic acid and linolenic acid, the potentially pro-inflammatory ω-6 PUFA, and less myristic, palmitic, and stearic acids. These long-chain SFAs are believed to have increased cardiovascular risk and can also have acute effects on cell growth and apoptosis. In MCT/LCT emulsions, MCT may be preferentially metabolized under certain clinical conditions and structured MCT may have a reduced tendency to accumulate in the reticulo-endothelial system. The olive oil-derived ω-9 MUFA appears less immunosuppressive and may inhibit release of pro-inflammatory cytokines. They are also less susceptible to peroxidation and well tolerated in critically ill neonates. Fish oil emulsions are predominantly ω-3 long-chain PUFA. Alone, they lack EFA and are formulated as a supplement to be administered with other nutritionally complete lipid products, or are manufactured as physical mixtures (10% fish:40% soy: 50% MCT or 30% soy:30% MCT:25% olive oil:15% fish). Fish oil-derived ω-3 PUFAs appear to alleviate symptoms of cholestasis, especially in neonates. Modern lipid products, based on olive, coconut, and/or fish oils, have demonstrable formulation and clinical benefits over traditional soybean and safflower IVLE and, when combined in the new multi-chamber bags, can also offer improvements in stability and safety [26–28].

47.3.1.3 Mineral Sources

In parenteral nutrition, calcium may be provided in the form of calcium gluconate, calcium chloride or calcium glycerophosphate. Due to aluminium contamination calcium gluconate was progressively abandoned by industry to meet the new FDA rule of 25 μg/L of aluminium in parenteral solution but remains frequently used in homemade hospital pharmacy preparations. Calcium chloride is easy to use but its high chloride content (2 mmol Cl^{-}/1 mmol Ca^{++}) limits its utilization in parenteral nutrition for VLBW infants [29, 30]. Calcium glycerophosphate with a 1:1 molar ratio is an adequate source of calcium and phosphorus, but is not registered for use in parenteral nutrition and needs to be prescribed from powdered anhydrous CaGlyP.

Phosphorus may be provided in inorganic (sodium or potassium phosphate) or organic form (glucose 1 phosphate, fructose 1-6 diphosphate, sodium glycerophosphate). Potassium phosphate is frequently preferred to sodium phosphate and used as the unique source of potassium in the parenteral solution. Potassium phosphate is easy to use but its high potassium content (1 mmol P^{3-}/1 mmol K for the monobasic form, 1 mmol P^{3-}/2 mmol K for the dibasic form or 1 mmol P^{3-}/1.7 mmol K for the mixed form) limit its utilization in parenteral nutrition for VLBW infants. Organic phosphorus in the form of disodium glucose 1 phosphate (Phocytan) is widely used in parenteral solution for VLBW infants. However, as for the use of sodium glycerophosphate, 2 mmol Na^{+}/1 mmol P^{3-}, or fructose 1-6 diphosphate (Esafosfina) the sodium content, 3 mmol Na^{+}/2 mmol P^{3-}, limits its utilization in VLBW infants particularly during the first weeks of life.

Magnesium is generally provided as magnesium sulfate in parenteral solution. Magnesium chloride has also been associated with the risk of inducing anionic-cationic imbalance in the parenteral solution.

47.3.1.4 Vitamins and Trace Elements

With the daily use of hydro- and lipo-soluble vitamins combined with trace elements clinical and biochemical evidence of deficiency were no longer reported.

47.3.2 Tailored or Standard Parenteral Solutions

Parenteral solutions can be prescribed using either of two formats: tailored or standard [31, 32]. Tailored solutions are formulated specifically to meet the daily nutritional requirements of the individual patient, whereas standard solutions are designed to provide a formulation that meets most of the nutritional needs of the stable biochemical and metabolic parameters. Both of these methods have advantages and disadvantages associated with their use.

Tailored solutions are based on the principle that no single parenteral regimen can be ideal for all patients, for a wide variety of pathological processes, all age groups, or for the

same patient during a single disease. The main advantage of tailored solutions is flexibility. Each solution is formulated for an individual patient and can be modified when the patient's nutritional needs and metabolic, electrolyte or clinical status changes.

The disadvantage of these solutions is linked to the time involved in calculation and label preparation, which today is nevertheless diminished with the use of specific computer programs. These solutions should be prepared with strict aseptic techniques, possibly in the pharmacy, not in the ward, and stored in a refrigerator at 4°C. The solutions thus prepared are stable for 96 hours and should be allowed to reach room temperature slowly and not warmed before infusion.

Standard solutions contain fixed amounts of each component per unit volume. In some hospitals there are a few types of fixed solutions to better cover the nutritional requirements of premature infants. The advantages of these solutions are that they include all the essential nutrients in fixed amounts, which eliminates the chances of inadvertent omission or overload. The disadvantage of standard solutions is their lack of patient specificity and the need of minimal adjustment particularly during the first days of life.

Very recently, it was suggested that the use of unique standard parenteral solution contribute to a significant improvement of nutritional support in both extremely and very preterm infants [33]. In addition, ready to use industrially manufactured multi-chamber bags (MCB) containing the 3 sterilized macro-nutrient solutions (amino acids, glucose, and lipids), in separate chambers of a single closed plastic system were evaluated in multicentric study and provides similar benefits [34]. Their guaranteed sterility and longer shelf life are major technological advances that minimize the risks of inadvertent contamination during compounding and storage.

47.3.3 Nutrient Intake

Table 47.4 shows the composition of a ready to use parenteral solution for VLBW infants used in our NICU and the daily nutrient intake (kg/d) given by total parenteral nutrition according to the new practice of "aggressive nutrition" for VLBW infants. In any case nutrient intakes are always indicative and may be modified according to each patient, his/her clinical picture, biochemical data and tolerance to nutrient intake. Thus, this standard solution could be diluted with free water according to fluid requirement and sodium intake could be adapted after a few days of life.

The following suggestions could be useful in the management of a parenterally fed VLBW infant.

- In the first days of life fluid intake should be increased if daily weight loss is > 5%, total weight loss> 12–15%, serum Na >150mmol/L, urinary osmolality >350 mOsm/L and if the infant is under phototherapy or managed in a radiant incubator. On the contrary fluid intakes should be reduced if daily weight loss <2% or there is a weight gain and serum Na <130 mmol/L.
- Glucose should be routinely administered after birth and progressively increased with the objective to increase energy intake. When a glucose infusion rate of 6 mg/kg/min or less leads to hyperglycemia, the use of insulin is advised. Insulin should be discontinued as soon as glucose tolerance is established and the energy supply necessary for growth can be delivered without hyperglycemia. If insulin is used, strict blood glucose monitoring is mandatory to avoid hypoglycemia.
- The starting dose of AA in the first day of life should be higher than what is currently advised. Intake should never

Table 47.4 Parenteral nutrition solution and daily nutrient intake (kg/d) of very low birth weight infants (<1500 g) in total parenteral nutrition according the "aggressive approach"

Day of life	Composition	D1 /kg/d	D2 /kg/d	D3 /kg/d	D4 /kg/d	D5 /kg/d	D6 /kg/d	>D6 /kg/d
Parenteral solution (mL)	100	50	70	100	120	140	150	150
Glucose (g)	12.5	6.3	8.8	12.5	15.0	17.5	18.8	18.8
Amino acid (g)	2.7	1.4	1.9	2.7	3.2	3.8	4.1	4.1
Calcium (mg)	72	36	50	72	86	100	108	108
Phosphorus (mg)	55	27	38	55	66	77	82	82
Magnesium (mg)	8	4.0	5.6	8.0	9.6	11.2	12.0	12.0
Natrium (mmol)	1.6	0.8	1.1	1.6	1.9	2.2	2.4	2.4
Kalium (mmol)	1.5	0.8	1.1	1.5	1.8	2.1	2.3	2.3
Chloride (mmol)	2.0	1.0	1.4	2.0	2.4	2.8	3.0	3.0
AA supplement (g) *		1.0	1.0	0.5	–	–	–	–
Lipid emulsion 20% (g) *		1.0	1.5	2.0	2.5	3.0	3.0	3.0
Total fluid (mL)		65	83	110	132	155	165	165
Total energy (kcal) **	57	42	69	76	91	108	113	113
Total AA g		2.4	2.9	3.2	3.2	3.8	4.1	4.1

* Provided separately in Y route, ** Energy (kcal) = AA × 3.75 + Glu × 3.75 + lip × 9.3.
These nutrient intakes are a mere indication and have to be modified according to the clinical picture and biochemical data of the single patient.

be less than 1.5 g/kg/d and the starting dose should preferably be 2.0–3 g/kg/d. When energy intakes reach about 70 kcal/kg/d and there is no enteral protein intake, the dose should be increased progressively to 3.5–4.4 g/kg/d.

- Although many neonatal intensive care units start lipid infusion only after the first days, it seems logical to start parenteral lipid within the first day in order to avoid a prolonged interruption of essential fatty acids and LC-PUFA. The starting dose of 0.5–1 g/kg/d should be gradually increased to 3–3.5 g/kg/d. The lipid infusion should be adjusted to maintain a serum lipid <250 mg/dL. Using a similar approach allows to reduce the cumulative nutritional deficit and limit or abolish postnatal growth restriction in VLBW infants [15, 32].

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48

Post-Discharge Nutrition in Preterm Infants

Richard J. Cooke

48.1 Introduction

The fundamental principle underlying nutritional support is that intake meets needs thereby ensuring the best outcome; in the case of the preterm infant, optimal growth and development. Achieving this goal is problematic and most, if not all very-low-birth-weight infants (VLBWI) are undernourished and under-grown at initial hospital discharge.

In term and preterm infants a clear relationship exists between poor growth and poor development. In preterm infants, the most rapid period of postnatal growth occurs between 37–38 weeks and 2–3 months corrected age. Infants who "recover" or "catch-up" during this period have better neurodevelopment while those who "fail to thrive" have poorer neurodevelopment. Precisely for these reasons preterm, particularly VLBWI, need close growth monitoring and specialized nutritional support after hospital discharge. In this chapter, we will review: a) the problem of undernutrition and postnatal growth failure (PGF) in preterm infants; b) the critical relationship between growth and development during infancy; c) studies examining growth and development in preterm infants fed nutrient-enriched formulas after hospital discharge; d) the breast fed infant; e) concerns about "recovery" or "catch-up" growth and the development of insulin resistance and visceral adiposity in preterm infants; f) outstanding issues about feeding preterm infants after hospital discharge.

48.2 Undernutrition and Postnatal Growth Failure in Preterm Infants

Several studies have examined postnatal growth in preterm infants during initial hospital stay and noted that most preterm, if not all very-low-birth-weight infants (VLBWI), are growth retarded at hospital discharge [1–7]. In studies where nutritional intake was measured, recommended dietary intakes (RDIs) took time to establish and infants accrued a significant nutrient deficit that was directly related to the growth deficit [1–5]. In studies where intake was not measured, poor growth was related to associated illness [6, 7]. This is not surprising because it takes time to establish RDIs in the smaller sicker infant. Once established, intake may be interrupted during episodes of clinical instability further increasing the nutrient deficit. However, other factors may underpin the development of growth failure in these infants.

Current recommendations are that the rate of weight gain parallel that of the fetus of the same gestational age once birth weight has been regained [8, 9]. Yet, a 26 weeks gestational age weighing ~ 850 g at birth who regains birth weight by 2 weeks of age and grows at the intrauterine rate will weigh ~550 g less than the fetus and be growth retarded at 36 weeks (Fig. 48.1). If current recommendations are met most, if not all, VLBWI will be growth retarded at hospital discharge.

Current recommendations assume that nutritional requirements are consistent throughout gestation for all preterm low birth weight infants [8, 9]. However, Ziegler et al have noted that protein and energy requirements change with advancing gestation [10] (Table 48.1). In effect, a formula containing a protein: energy ratio of 3.0 g/100 kcal, as is currently fed to preterm infants, does not meet the protein: energy needs of the infants weighing ≤1500 g at birth; i.e., the VLBWI.

Recommended dietary intakes are based on needs for maintenance and normal growth [8, 9], no allowance is made for recovery or "catch-up" growth [11]. In the study of Embleton et al, the accrued protein deficit at hospital discharge varied from 15 to 25 g/kg [3]. An additional protein intake of 0.5–1.0 g/kg/day would have been required to recoup this deficit before hospital discharge, further compounding the problem.

To further examine this issue, body size and body composition were measured in preterm infants at hospital discharge [12]. Weight, length and head circumference were measured using standard methodology [13]. Body

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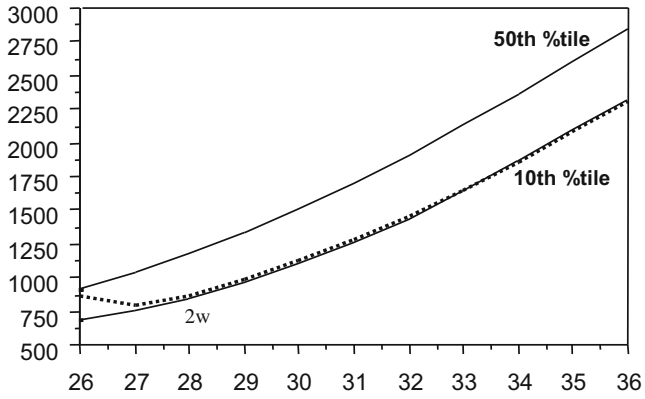

Fig. 48.1 Growth curve for a 26 weeks preterm infant who regains birth weight by 14 days and grows at a rate that parallels that in utero

Table 48.1 Protein and energy needs in preterm infants

Weight (g)	500–700	700–900	900–1200	1200–1500
Protein (g/kg/day)	4.0	4.0	4.0	3.9
Energy (kcal/kg/day)	105	108	119	127
P:E (g/100 kcal)	3.8	3.7	3.4	3.1

composition was measured using dual-emission X-ray absorptiometry (DEXA) [14].

One hundred and forty nine infants (birth weight = 1406 ± 248 g, gestation = 31 ± 1.7 weeks) were studied. Post-menstrual age at discharge was 37 ± 1.2 weeks. However, z-score for head circumference was significantly greater than that for weight which in turn was greater that for crown-heel length ($-0.1 \pm 0.6 > -1.4 \pm 0.6 > -1.9 \pm 0.6$; $p < 0.0001$) [15]. Body composition results are compared to the reference infant at the same weight and same gestation in Table 48.2.

Global fat-free mass was less in study infants than the reference infant at the same weight (2062 < 2252 g; $p < 0.0001$) or gestation (2062 < 2667 g; $p < 0.0001$). Global fat mass was greater in study infants than the reference infant at the same weight (307 > 198 g, 13 > 8%) or gestation (307 > 273 g; 13 > 9%; $p < 0.0001$). At the same time, changes in central fat mass closely paralleled those in total fat mass ($y = 23 + 0.31x$, $r^2 = 0.77$, $p < 0.0001$) [12].

Table 48.2 Body composition of study infants compared to the reference infant at the same weight and same gestation

	Study infants	Reference at 35 weeks	Reference at 37 weeks
Body weight (g)	2369 ± 305	2450	2940
Fat-free mass (g)	2062 ± 277*	2252*	2667*
Fat-free mass (%)	87 ± 3.4	91.9	90.7
Fat mass (g)	307 ± 130**	198**	273**
Fat mass (%)	13 ± 3.4**	8.1**	9.3**

* Study infants < Reference infant at 35 or 37 weeks ($p < 0.0001$).
** Study infants > Reference infant at 35 or 37 weeks ($p < 0.001$).

A reduced body size that is paralleled by reduced linear growth and a reduced fat-free mass suggest that dietary protein needs were not met in these infants before hospital discharge, as has been previously suggested [10]. A reduced fat-free mass coupled with an increased global and central fat mass echoes concerns about visceral obesity and the development of insulin resistance in these high-risk infants [16, 17].

Although many factors contribute to the development of postnatal growth failure and the degree of failure will vary, depending upon the level of maturity of the infant [3], one point is clear; i.e., close attention must be paid to nutritional support and growth of these infants after hospital discharge. A reduced body size that is paralleled by a reduction in fat-free mass but a relative increase in fat mass indicate that particular attention be paid to dietary protein and energy intakes in these infants.

48.3 Growth and Development in Preterm Infants

Nutrients play a critical role the promotion of normal health and prevention of disease [18]. It is not surprising, therefore, that malnutrition can be directly related to alterations in organ structure and function which, in turn, are paralleled by an increased morbidity and mortality in adults and children [18]. However, the effects of malnutrition appear to be greater during early infancy than later on in life. There are several reasons for this. Requirements are a function of growth rate, the greater the rate the greater the requirement, the more likely that deficiency will occur. Growth rates are greater during infancy than later in life; i.e., the term infant will double birth weight by 4–5 months, triple birth weight by 12 months and approximately quadruple it by 24 months.

Studies have also suggested that growth is "pre-programmed" to occur at a certain time or "critical" epoch which if missed may not be recoverable [19]. In effect, even short periods of nutritional deprivation may not only affect somatic but also brain growth and development [19], the area of the brain that is "programmed" to grow fastest being the most affected [20].

Studies in term infants have shown that malnutrition during infancy is associated with permanent alterations in brain growth and function. Brain size is reduced [21–25], the brain cortex is thinner [26], neuronal numbers are decreased [27], myelination is reduced [28] and dendritic morphology is altered [29, 30], all of which can be related to poorer neuro-developmental outcome [31–39].

Concerns for the term are even greater in preterm infants. Growth rates are greater. A preterm infant is "programmed" to quadruple brain weight between 24 and 40 weeks gestation or 16 weeks [40], almost 6 times faster than the term infant. Preterm infants are more vulnerable to the effects of perinatal ischemia and inflammation, therefore the development of

periventricular intraventricular hemorrhage and periventricular leucomalacia [41]. They are also more likely to be fetally and/or postnatally malnourished. Up to 40% of preterm infants are growth retarded or small for gestational age (SGA) at birth [42], while up to 100% of VLBWI are SGA at hospital discharge [6].

Poor fetal growth is paralleled by reduced organ growth as well as altered structure and function [43] but not all organ systems are affected equally. This is nicely illustrated in the study of Myers et al [44]. In this study, 30% reduction in body weight in SGA monkeys was associated with an 8% reduction in brain weight but ≥35% reduction in lung, liver, pancreatic and spleen weights when compared to their AGA counterparts [44]. Thus, the brain is "spared" at the expense of other organs which; e.g., through the development of chronic lung disease, sepsis, etc [6], may amplify undernutrition by reducing intake and increasing requirements.

In the late 80's and early 90's, studies indicated that poor growth between birth–hospital discharge was associated with poorer neurodevelopment [45, 46] and that better growth, as achieved by feeding a nutrient-enriched formula, was associated with better developmental outcomes [47, 48]. More recently, early parenteral nutrition coupled with the early introduction and advancement of enteral feeds has been associated with better growth but many infants continue to be SGA at hospital discharge [49–52].

A clear relationship exists between "catch-up" growth and development in preterm infants but the time frame within which it needs to occur is not well delineated. In most studies, infants who "recover" or "catch-up" by 6–9 months corrected age (mca) have better neuro-developmental outcome [45, 53–56]. This is nicely illustrated in the study of Latal-Hajnal et al [56]. In this study, postnatal growth and neurodevelopment were studied in 219 VLBWI. Infants were stratified at birth into those who were appropriate size for gestational age (AGA weight ≥10th %tile) and SGA (weight <10th %tile). This process was repeated at 2 y of age. Four groups emerged: a) AGA at birth and 2 yrs (AGA-AGA), b) AGA at birth and SGA at 2 yrs (AGA-SGA), c) SGA at birth and AGA at 2 yrs (SGA-AGA) and d) SGA at birth and 2 yrs (SGA-SGA).

No differences were noted in development between the AGA-AGA and SGA-SGA infants. After adjusting for co-variables, infants who "caught-up"; i.e., SGA-AGA, had greater Bayley PDI (Psychomotor Development Index) scores than those who did not; i.e., SGA-SGA. Infants who "faltered"; i.e., AGA-SGA, had lower PDI's and MDI (Mental Development Index) scores, than those who continued to thrive; i.e., AGA-AGA. These authors concluded that the course of postnatal growth primarily determined later development.

To further examine this issue, our group examined postnatal growth and development in 119 preterm infants with a gestational age of <32 weeks [57]. It was hypothesized that the greater the degree of growth failure between birth–28 days; i.e., fall in z-score between birth and 28 days, the poorer the development at 18 mca. Body weight was determined at birth, 28 days, hospital discharge and serially until 18 mca. Developmental outcome was determined at 18 mca.

At 28 days, infants were stratified into those who were mildly (fall in z-score <–1.0 SD; MGR), or severely (fall in z-score ≥ –1.0 SD; SGR) growth retarded. At 18 mca, this process was repeated and 4 groups emerged; mildly growth retarded at 28 days and 18 mca (MGR-MGR), mildly growth retarded at 28 days but severely growth retarded at 18 mca (MGR-SGR), severely retarded at 28 days but mildly retarded at 18 months (SGR-MGR), severely retarded at 28 days and 18 mca (SGR-SGR).

The characteristics of the study infants are presented in Table 48.3. No differences were noted with the exception that gestational age was less (SGR-SGR < MGR-MGR, MGR-SGR, $p < 0.05$) and the frequency of cerebral palsy tended to be greater (SGR-SGR >MGR-MGR, SGR-MGR, $p < 0.10$) in infants with severe growth failure at 28 days and 18 mca.

Growth of the study infants is presented in Fig. 48.2. Between birth–28 days, all infants failed to thrive. Thereafter, all infants "recovered" or "caught-back" to some degree. However, recovery was more complete in the MGR-MGR and SGR-MGR than in the MGR-SGR and SGR-SGR infants. Infants who "recovered" (SGR-MGR) or "faltered", (MGR-SGR) did so between 28 days and 1–2 mca, a time when programmed growth velocity is greatest in the preterm infant [58].

Bayley's developmental scores are presented in Table 48.4. A 17-point difference in MDI ($p < 0.01$) and 14-point difference in PDI ($p < 0.05$) was noted between the MGR-MGR when compared to SGR-SGR infants supporting the hypothesis

Table 48.3 Characteristics of study infants

Group (N)	MGR-MGR (50)	MGR-SGR (18)	SGR-SGR (16)	SGR-MGR (24)
Birth weight (g)	1320 ± 339	1348 ± 387	1312 ± 559	1271 ± 408
Gestation (weeks)	30 ± 1.6*	30 ± 1.8*	28 ± 3.0*	29 ± 2.3
BPD	13 (26%)	3 (17%)	6 (38%)	10 (42%)
Abnormal CUS	10 (20%)	5 (28%)	3 (19%)	6 (25%)
PVL	3 (6%)	2 (11%)	1 (6%)	3 (13%)
Cerebral palsy	4 (8%)	3 (17%)	5 (30%)**	2 (8%)

* SGR-SGR < MGR-MGR, MGR-SGR, $p < 0.05$.
** SGR-SGR > MGR-MGR, SGR-MGR, $p < 0.10$.

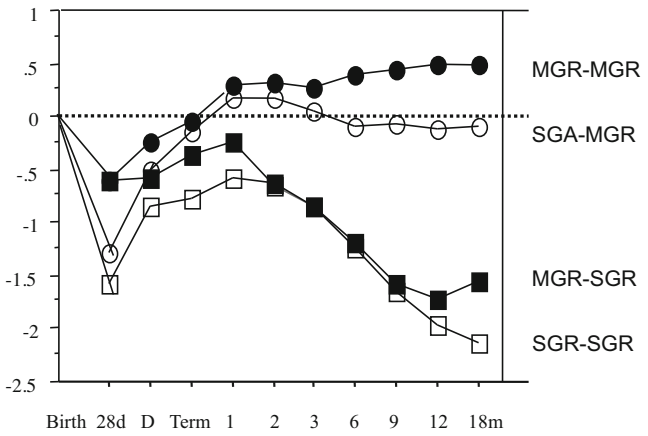

Fig. 48.2 Growth, i.e., change in z-score between birth and 28 days, term and one through 18 months corrected age, in study infants

Table 48.4 MDI and PDI in study infants

Group (N)	MGR-MGR	MGR-SGR	SGR-MGR	SGR-SGR
MDI*	93 ± 18	91 ± 18	95 ± 1	77 ± 19
PDI*	90 ± 17*	83 ± 19	87 ± 19**	77 ± 22*
MDI**	96 ± 15	93 ± 20	98 ± 16	88 ± 11
PDI**	94 ± 13	89 ± 15	91 ± 14	89 ± 16

* MDI ~ MGR-MGR, SGR-MGR > SGR-SGR. p < .05.
* PDI ~ MGR-MGR (p <.05), SGR-MGR (p <.10) > SGR-SGR.
** Infants with cerebral palsy excluded. MDI ~ MGR-MGR (p < .05), SGR-MGR (p < .10) > SGR-SGR.

that the greater the degree of growth faltering between birth–28 days, the poorer the developmental outcome. An 18-point difference was noted in MDI (p <0.05) and a 14-point (p <0.10) difference in PDI between SGR-MGR and SGR-SGR infants. When infants with cerebral palsy are excluded, differences in MDI but not PDI persist between the study groups.

Collectively, these data support the conclusions of Latal-Hajnal et al; i.e., it is the course of postnatal growth that determines later developmental outcome. These data also support the idea that a "critical window of opportunity" exists between 28 days and 1–2 mca during which "recovery" or "catch-up" growth to the original birth weight %tile is paralleled by better neuro-developmental outcome. If infants are to "recover", this is the time to do it.

48.4 Studies Examining Post-Discharge Nutritional Support in Preterm Infants

48.4.1 Effects on Growth

Several studies have examined post-discharge growth in preterm infants [36, 59–64]. Although some "catch-up" growth has been observed, preterm infants do not grow as well as their term counterparts and are smaller at 3 y [65], 8 y [66] and adulthood [67]. There are several possible reasons for this.

Current in-hospital feeding practices ensure that most, if not all VLBWI are undernourished and growth retarded at initial hospital discharge [3, 7]. A "critical epoch" of growth may, therefore, have been missed. Preterm infants also have greater morbidity than term infants during the first year of life [68–72] and inter-current illness will affect growth, irrespective of whether infants are admitted to hospital or not.

Until relatively recently, little attention had been paid to nutritional factors in the pathogenesis of this problem. For most early studies, infants were fed either human milk or a term infant formula after hospital discharge [36, 59–64]. Both feeding regimens were designed to meet nutritional needs of the term rather than the rapidly growing preterm infant. Infants, therefore, may have been partly underfed during the first 6–12 months of life.

More recently studies have examined the effects of feeding nutrient-enriched formulas; i.e., formulas with a greater nutrient density than a term infant formula, to preterm infants after hospital discharge [13, 14, 73–83]. The general characteristics of these studies are listed in chronologic order in Table 48.5.

Lucas et al randomized preterm infants (birthweight ≤ 1800 g, gestational age ≤ 34 weeks; n =16/gp) to be fed either a term formula or nutrient-enriched infant formula after hospital discharge. Those fed the nutrient-enriched formula grew better [73] and had better bone mineralization at 3 and 9 mca [74]. However, Chan et al were unable to show any differences in growth between preterm infants fed a term formula and those fed either a nutrient-enriched or preterm infant formula after hospital discharge [75].

Cooke et al randomized otherwise "normal" preterm (≤1750 g birth weight, ≤34 weeks gestation) infants to one of three feeding groups: one group (n = 49) was fed a nutrient-enriched infant formula between discharge–6 mca, the second group (n = 54) a term formula between discharge–6 mca and the third (n = 26) a nutrient-enriched formula between discharge–erm and a term formula between term–6 mca [13, 14, 77].

Infants fed the nutrient-enriched formula between discharge–6 mca, had lower volumes, similar energy but greater protein intake than the other groups (Fig. 48.3). Increased protein intake was paralleled by greater serum urea nitrogen and weight and length gains, which in turn, were reflected by greater body weight, length, lean mass and absolute (g) but not fractional fat (%) mass. No other differences were detected in growth or body composition between the groups. Initial analyses indicated that effects on growth were predominantly in boys. A subsequent analysis was performed when data were converted to z-scores (Fig. 48.4). Between birth and discharge, z-scores for body weight fell in boys in both treatment groups. Between discharge–6 mca, z-scores were consistently greater in boys fed the preterm formula. The pattern is the same in girls, indicating that girls also benefited when fed the nutrient-enriched formula.

Table 48.5 Characteristics of studies examining growth in preterm infants fed nutrient-enriched formulas after hospital discharge

Study	Protein content*	Energy content**	Sample size	Study duration	Benefit
Lucas et al [73, 74]	2.6 g	72 kcal	32	9 mca***	Yes[1]
Chan et al [75]	2.6 g	67 kcal	43	16 wca****	No
Wheeler et al [76]	2.7 g	67 kcal	43	12 wca	Yes[2]
Cooke et al [13, 14, 77]	2.8 g	80 kcal	129	6 mca	Yes[3]
Lucas et al [78]	2.6 g	72 kcal	229	9 mca	Yes[4]
Carver et al [79]	2.6 g	74 kcal	125	12 mca	Yes[5]
De Curtis et al [83]	2.4 g	74 kcal	33	2 mca	No
Agosti et al [80]	3.0 g	80 kcal	121	15 wca	Yes[6]
Lapillonne et al [81]	2.7 g	81 kcal	37	Term	Yes[7]
Koo et al [82]	2.6 g	74 kcal	89	12 mca	No

* Study formula /100 kcal ** Study formula /100 mL *** Months corrected age **** Weeks corrected age
[1] Increased weight and length at 9 months.
[2] Increased length and head circumference at 8 wca.
[3] Increased weight, length and head circumference at 6, 12 and 18 mca, predominantly in boys.
[4] Increased length at 18 months, boys only.
[5] Increased weight, length and head circumference at 12 months in infants weighing < 1250 g at birth, predominantly in boys.
[6] Increased weight at 6 months and 6 mca, boys only. Increased length at 12 mca, SGA infant boys only.
[7] Increased weight and bone mineral content at term.

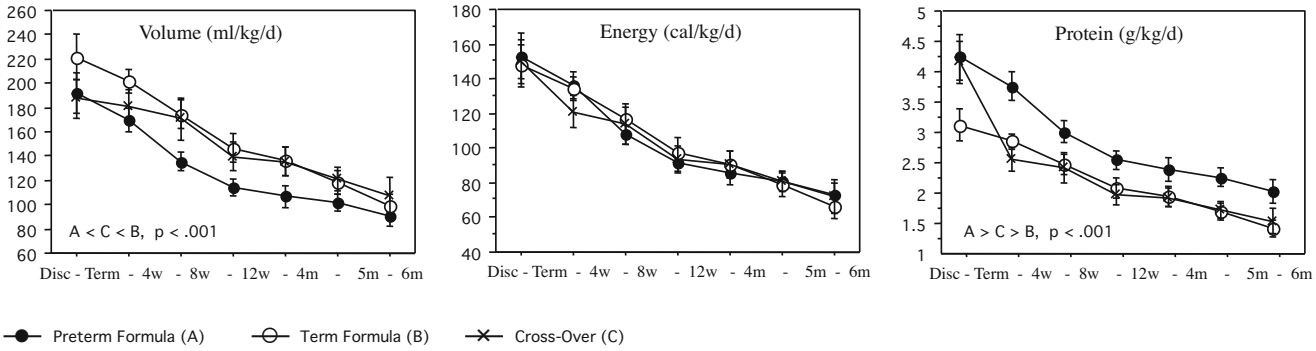

Fig. 48.3 Nutrient intakes between discharge and 6 months in study infants fed either a preterm infant formula (group A), a term formula (group B) or a preterm infant formula (discharge-term) and term infant formula (term–6 months; group C)

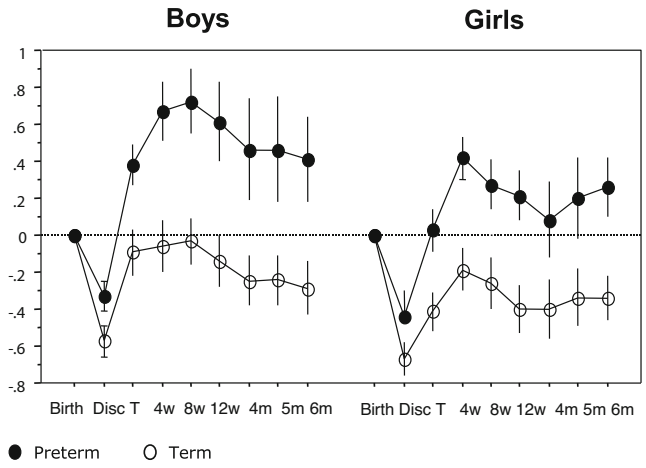

Fig. 48.4 Growth, expressed as weight z-score, in boys and girls fed either the preterm formula or the term infant between discharge and 6 months corrected age

Carver et al randomized preterm infants (<37 weeks, ≤ 1800 g) to be fed either a nutrient-enriched formula (n = 67) or a term formula (n = 56) between discharge and 12 mca [79]. Infants were stratified according to birth weight (< 1250 g, ≥ 1250 g). No differences were detected in energy intake but protein intake was greater in infants fed the nutrient-enriched formula. Growth was also better, particularly in infants <1250 g who were heavier, longer and had a greater head circumference at 6 mca.

Lucas et al randomized a group of preterm infants (< 37 weeks, < 1750 g) to be fed a either a nutrient-enriched (n = 113) or term (n = 116) formula between discharge and 9 mca [78]. Infants fed the enriched formula grew better; i.e., were heavier and longer at 9 mca and longer at 18 mca, than infants fed the term formula, an effect that was more marked in boys than girls.

De Curtis et al randomized a group of preterm infants (< 35 weeks gestational age; birth weight of < 1750 g) to be

fed either a nutrient-enriched formula (n = 16) or a standard term formula (n = 17) between hospital discharge at ~ 37 weeks and ~ 2 mca [83]. No differences were detected in volume of formula intake but protein and energy intakes were greater in infants fed the nutrient-enriched formula. No differences were detected in growth or body composition between the treatment groups.

Agosti et al randomized preterm infants (< 1500 g) to be fed either a nutrient-enriched (n = 62) or a standard term infant (n = 52) formula between term and 15 weeks corrected age (wca) [80]. A trend towards to increased weight and head circumference was noted at 12 mca, while weight increase was greater in boys at 15 wca and 6 mca in infants fed the nutrient-enriched formula. Length increase at 12 months was also greater in SGA infants fed the nutrient-enriched formula.

Lapillone et al randomized preterm infants (28–32 weeks gestation; 1000–1500 g) to be fed either a standard (n = 20) or a nutrient-enriched (n = 21) preterm formula during initial hospital stay until term corrected age, and then to ~ 3 mca [81]. The formulas differed in protein (2.5 v 2.7 g/100 kcal) and mineral content, plus 25% and 40% for calcium and phosphorus intake, for infants fed the nutrient-enriched formula. No differences were detected in growth but bone mineral mass was greater at term in infants fed the latter.

Koo et al randomized preterm infants (630–1620 g; 24–34 weeks gestation) to be fed a nutrient-enriched (n = 45, protein content 2.6 g/100 kcal) or term (n = 45, protein content 2.14 g/100 kcal) between hospital discharge and 12 mca [82]. Weight, length and head circumference were greater in infants fed the term infant formula. Lean, fat and bone mass were also greater in these infants.

Reviewing the results of studies published before 2005 Henderson et al concluded that feeding with nutrient-enriched formulas had little effect on growth [84]. As such, their conclusions on growth merit examination.

The first outcome variable evaluated was "growth during the trial period". The results of only one study were used; i.e. de Curtis et al [83] wherein the sample size was 33 and the end-point was gain in weight, crown-heel length and head circumference between 36 wca and 2 mca. Yet, growth velocity changes rapidly during this time (Fig. 48.5) and that which is averaged over a 12 weeks period may not reflect early but significant differences between the groups [13].

The second end-point was "longer-term growth"; i.e., weight, length and head circumference at 6, 9 and 18 mca. Data from only one study was used at 6 mca [85] and one at 9 mca [78], again not entirely representative. The conclusions drawn by Henderson et al on growth, therefore, must be questioned. However, a closer look at these studies is revealing.

In the study of Cooke et al, one group was fed the nutrient-enriched formula to term and no growth advantage was detected [13, 14, 77]. In the study of de Curtis et al, the nutrient-enriched formula was fed to 2 months and no growth advantage was detected [83]. Yet, in the studies with an adequate sample size, where the nutrient-enriched formulas were fed to 6 [13, 14, 77], 9 [78] and 12 [79] mca, growth was better, suggesting that duration of feeding is an important consideration.

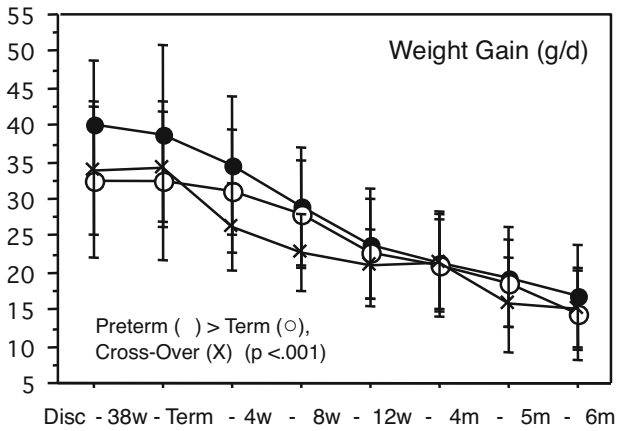

Fig. 48.5 Growth velocity, expressed as weight gain, in study infants fed either a preterm infant formula (*closed circle*), a term formula (*open circle*) or a preterm infant formula (discharge-term) and term infant formula (X)

In the studies of Cooke et al [13, 14, 77], Carver et al [79] and Lucas et al [78] infants were stratified according to sex. In these studies, the effect of diet was greatest in males. This may not be surprising because boys are programmed to grow faster and accrete more lean mass [86] and, therefore, benefit from a higher protein-to-energy ratio. This is illustrated in Fig. 48.6 where boys fed the nutrient-enriched formula had greater weight gain, lean mass gain, body weight and lean mass when compared to girls fed the same formula [14].

In the study of Carver et al infants were stratified according to birth weight, <1250 and 1250–1800 g [79]. Infants < 1250 g also seemed to benefit more from the nutrient-enriched formula. This also is not surprising. At hospital discharge, the more immature the infant the greater the accrued nutrient deficit the more likely the infant is to benefit from a post-discharge nutrient-enriched formula.

48.4.2 Effects on Development

Three studies have examined neuro-developmental outcome in preterm infants fed a nutrient-enriched formula after discharge in otherwise "normal" preterm infants [77, 78, 80] with another study assessing outcome in infants with perinatal brain injury [87].

Cooke et al also fed preterm infants (n = 113, ≤ 34 weeks gestation) either a nutrient-enriched or a term formula between discharge–6 mca [77, 78]. Although boys fed the nutrient-enriched formula had a greater head circumference at 6, 12 and 18 mca no differences were noted in MDI or PDI between the treatment groups (Table 48.6). However, boys

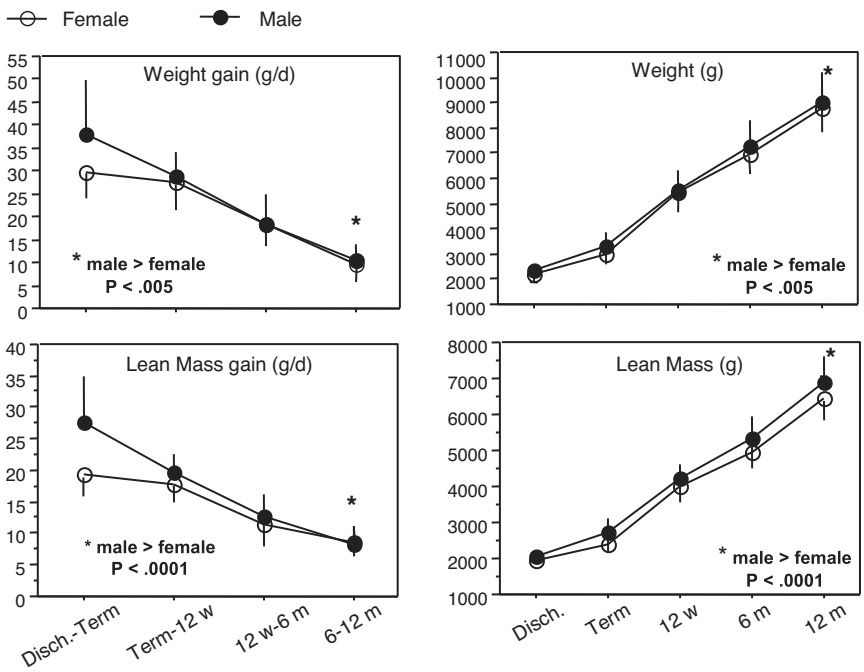

Fig. 48.6 Growth and lean body mass between discharge and 6–12 months corrected age in boys and girls fed the preterm infant formula

fed the term formula had the lowest head circumference [77]. Boys fed the term formula also had an MDI that was 10 points lower than girls fed the term formula (Table 48.6), suggesting that they were at a disadvantage when fed the term formula.

In the study of Lucas et al, preterm infants (n = 229, < 37 weeks gestation) were fed either a nutrient-enriched or a standard term infant formula between discharge–9 mca [78]. At 9 mca, no differences were noted in head circumference or development. At 18 months, no differences were noted in head circumference or development but infants fed the nutrient-enriched formula had a + 3 advantage point in PDI and it was concluded that they "could not reject the hypothesis that post-discharge nutrition benefits motor development" [78].

Agosti et al fed preterm infants (n = 121) either a nutrient-enriched (protein content = 3.0 g/100 kcal; energy content = 80 kcal/100 mL) or a term (protein content = 2.4 g/100 kcal, 70 kcal/100 mL) infant formula between term and 15 wca [80]. Development at 6 months, as assessed on the Griffiths Developmental Scale, was better in small for gestational age boys fed the nutrient-enriched formula.

Table 48.6 Bayley MDI and PDI scores in study infants

Formula group	Nutrient-enriched	Term	Nutrient-enriched + Term
MDI			
1. All	102 ± 14	103 ± 14	102 ± 11
2. Girls	103 ± 14	107 ± 14	104 ± 12
3. Boys	100 ± 15	97 ± 11	99 ± 11
PDI			
1. All	102 ± 8	103 ± 9	99 ± 8
2. Girls	103 ± 8	105 ± 9	98 ± 8
3. Boys	101 ± 7	101 ± 7	101 ± 7

Some insights can be obtained by comparing these three studies [77, 78, 80]. Although enrollment criteria and composition of studies formulas differed significantly between the studies and no overall differences were noted in development between control and treatment groups, all suggest that male infants are more likely to benefit from being fed a nutrient-enriched formula. Put in another way, male infants appear to be at a disadvantage when fed a term formula after hospital discharge.

More recently, Dabydeen et al prospectively randomized term and preterm infants with perinatal brain injury to either a control or high energy and protein diet during the first year of life [87]. Infants fed the high energy and protein diet had a greater head growth and axonal diameters when compared to the control group. It was concluded that infants with significant perinatal brain injury had increased nutritional requirements and that inadequate intake, as is commonly noted in neurologically impaired infants, may compromise subsequent brain growth and development [87].

Collectively, these data [77, 78, 80, 87] suggest that post-discharge nutrition may affect developmental outcome. In the case of the otherwise "normal" preterm infant, male infants appear most likely to benefit. In infants with perinatal brain injury, both term and preterm infants may benefit. Further appropriately "powered" studies are needed to examine both these issues.

48.5 The Breast-Fed Infant

Before hospital discharge, preterm infants fed human milk do not grow as well as infants fed nutrient-enriched formulas

[88, 89]. It is, therefore, recommended that human milk be fortified with additional nutrients [9]. Growth improves but it is still not as good as in infants fed a preterm infant formula [2]. The reasons for this are not entirely clear.

Fortifiers differ in nutrient composition and it is unclear which, if any, really meets requirements. The composition of human milk varies widely [90] and because it is not consistently measured there is no way of knowing what the infant is really receiving. In effect, intake less adequately meets requirements and growth is poorer. Nonetheless, because of lower rates of morbidity; e.g., sepsis and necrotizing enterocolitis [91], and improved developmental outcome [92] it is recommended that fortified human milk be fed whenever possible before hospital discharge [9].

After hospital discharge, breast-fed infants also grow more poorly than those fed nutrient-enriched formulas [75, 78, 93]. This also is not surprising. Before discharge, intake less adequately meets requirements, the accrued nutritional deficit and, therefore, needs for "recovery" are greater. Mature human milk is designed to meet the needs of the term infant and not the preterm infant and there are significant differences in nutrient content between mature human milk and formulas used to feed infants before and after discharge (Table 48.7).

Poorer growth after discharge is paralleled by alterations in bone mineral metabolism and body composition. Bone mineral accretion is less in infants fed unsupplemented human milk after discharge [75, 78, 93–95]. This, in turn, can be related to outcome on a short-term; e.g., osteopenia [96] and fractures [97], and long-term; e.g. poorer linear growth [98], basis. Infants fed human milk also have increased fat accretion when compared to those fed a nutrient-enriched formula [93].

These data support the idea that infants grow as they are fed. When mineral intake is inadequate infants are at-risk for deficit in mineral accretion. If the protein–to–energy intake is inadequate then, as in term infants [99], infants will accrete less lean but increased fat mass. Whether the latter in some way alters "programming" and, therefore, health in adult life remains to be determined.

In a recent stratified and randomized controlled pilot trial, preterm infants (750–1800 g) were fed unfortified (n = 20) or fortified (n = 19) human milk after hospital discharge–12 weeks [100]. A multi-nutrient fortifier, estimated to ensure an energy and protein density of 80 kcal and 2.2 g/100 mL, was added to 50% of feeds. Weight tended to be greater (all) while length (all) and head circumference, for infants ≤ 1250 g only, were greater in the fortified group. These data tend to support the idea that fortification, at these levels, promotes a proportional increase in lean and bone mass but further studies are needed in a larger group of infants to determine the best method to fortify human milk in these high-risk infants.

In the meantime, follow-up care and advisement of breast-feeding mothers after hospital discharge remains a critical issue. How often should these high-risk infants be followed up? What are acceptable growth rates and biochemical determinations in breast-fed infants? Schanler has suggested that human milk be fortified when weight, length or head circumference gains are < 25 g/day, 1.0 cm/weeks and 0.5 cm/weeks and or serum phosphorus, alkaline phosphatase or urea nitrogen levels are < 4.5 mg/dL (< 1.45 mmol), > 450 IU/L and <0.5 mg/dL (0.8 mmol) in the first 6–8 weeks after hospital discharge [101]. Given the relatively rapid rates of programmed growth [58] and critical importance that "catch-back" plays on brain growth and development during this period [57] infants should be seen every 2–3 weeks between hospital discharge and 3 mca.

Table 48.7 A comparison of nutrient content of human milk with preterm, nutrient-enriched post-discharge and term infant formulas

Per liter	Human milk	Preterm	Post-discharge	Term
Energy (kcal)	670	810	730	670
Protein (g)	10	23	20	14
Fat (g)	35	42	40	36
Carbohydrate (g)	70	88	78	73
Calcium (mg)	260	1400	850	530
Phosphorus (mg)	140	700	480	330
Sodium (mmol)	9	15	11	8
Chloride (mmol)	16	19	17	12
Zinc (mg)	3.2	12.1	9	6
Vitamin A (mg)	0.7	3	1	0.6

48.6 Catch-up Growth, Insulin Resistance and Visceral Obesity in Preterm Infants

Concern has been expressed about "catch-up" growth and the subsequent development of insulin resistance [102, 103] and metabolic syndrome X [104] leading to the idea that "bigger might not be better", even in preterm infants [103]. However, a certain amount of confusion exists when the term "catch-up" is used to describe growth during infancy.

In many instances, "catch-up" growth is related to weight gain and then to the subsequent risk of obesity [104]. It has been interpreted more as a pathologic rather than physiologic phenomenon and, therefore, best avoided. But weight gain "per se" reveals little information on the nature of the gain. It also fails to recognize that it is not preventable; i.e., after a period of growth faltering all infants "recover" or "catch-up" to some degree once the underlying cause is treated and more adequate nutrition is provided.

The extent to which "catch-up" occurs depends on many factors, including timing of the insult. Growth is thought to be programmed within a specific time frame or critical epoch which if missed my not be recoverable [43, 105]. It also depends upon the severity and duration of the insult, the more severe and prolonged the insult the greater the accrued nutritional deficit. Subsequent intake must not only replace the accrued nutritional deficit but also meet needs for maintenance and normal growth.

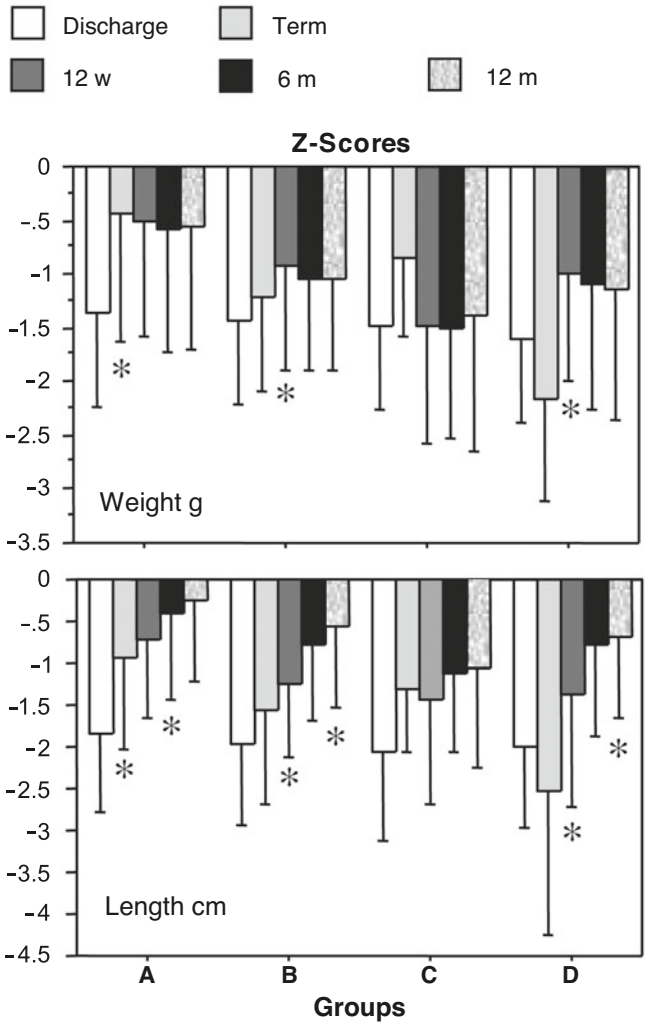

Fig. 48.7 Growth, expressed as weight and length z-scores, in study infants fed either a preterm infant formula (A), a term formula (B) or a preterm infant formula (discharge-term) and term infant formula (C) compared to a reference group of breast-fed infants (D)

With acute illness, infants develop a hyper-metabolic state and there is an acute depletion of body protein to meet energy needs [106]. With chronic protein-energy malnutrition during infancy visceral tissue is conserved at the expense of protein and fat [107]. Nutritional rehabilitation in these infants with high-energy intakes results in increased weight gain [108] and body fat [108, 109]. In effect, the nature of the gain is directly dependent upon the compositional nature of intake.

At hospital discharge, preterm infants have accrued a significant protein deficit [3] and are lighter, shorter and fatter that the reference infant at the same body weight or gestation [16]. After hospital discharge, a diet that is relatively high in energy may promote "catch-up" that is paralleled by increased and altered adiposity; i.e., central fat accretion. A diet that better meets protein needs may be paralleled by increased lean mass accretion.

Term infant formulas have a protein content of 1.8 g/100 kcal. Nutrient-enriched formulas, as fed to preterm infants after hospital discharge, have a protein content that varies from 2.5 to 2.7 g/100 kcal. In a prospective randomized controlled trial (Fig. 48.7), body size and composition were measured in preterm infants fed either a preterm formula (protein content 2.7 g /100 kcal, discharge–6 months; A), a term formula (protein content 1.8 g/100 kcal, discharge–6 months; B), a preterm formula to term then a term formula to 6 months; C) or unfortified breast milk (D).

"Catch-up" in weight and length were faster and more complete in infants fed the preterm formula (group A; Fig. 48.7). This was paralleled by an increase in non-fat mass and total fat mass but not % fat mass (Table 48.8). Changes in central fat mass (trunk + pelvis; $y = 41 + 0.30x$, $r^2 = 0.90$, $p < 0.0001$) and leg fat mass ($y = 034 + 0.40x$, $r^2 = 0.91$, $p < 0.0001$) were linearly related to those in total fat mass. Changes in central and leg fat mass accounted for 30% and 45% of the variation in total fat mass. No differences were detected in central fat mass between the groups but leg fat mass was greater in infants fed

Table 48.8 Body composition in study infants

	Group	Term (n = 148)	12 weeks (n = 141)	6 months (n = 145)	12 months (n = 138)
Fat-free mass* (g)	Preterm (A)	2745 ± 445	4270 ± 452	5208 ± 638	6872 ± 806
	Term (B)	2393 ± 276	4022 ± 411	5139 ± 515	6592 ± 738
	Cross-Over (C)	2507 ± 244	3948 ± 431	4978 ± 541	6399 ± 881
	Breast-Fed (D)	2171 ± 296	3762 ± 1051	5063 ± 568	6451 ± 746
Fat mass** (g)	Preterm (A)	570 ± 256	1455 ± 461	2033 ± 686	2332 ± 679
	Term (B)	511 ± 222	1367 ± 419	1940 ± 586	2058 ± 477
	Cross-Over (C)	566 ± 204	1188 ± 366	1815 ± 632	2077 ± 623
	Breast-Fed (D)	331 ± 128	1365 ± 527	1934 ± 658	2153 ± 645
Fat mass (%)	Preterm (A)	17 ± 5.5	25 ± 4.7	28 ± 5.9	25 ± 5.3
	Term (B)	17 ± 6.0	25 ± 5.5	27 ± 5.1	24 ± 4.4
	Cross-Over (C)	18 ± 4.7	23 ± 4.1	26 ± 5.8	24 ± 4.5
	Breast-Fed (D)	13 ± 3.2	25 ± 8.2	27 ± 6.8	25 ± 4.8

* A > B ($p < .05$), C ($p < .001$), D ($p < .005$).
** A > B ($p < .05$), C ($p < .005$).

Table 48.9 Regional fat accretion (grams) in study infants

	Group	Term	12 weeks	6 months	12 months
Trunk	Preterm (A)	172 ± 65	532 ± 168	651 ± 218	699 ± 249
	Term (B)	182 ± 71	485 ± 154	635 ± 170	613 ± 160
	Cross-Over (C)	186 ± 67	394 ± 136	579 ± 221	573 ± 162
	Breast-Fed (D)	147 ± 54	503 ± 183	688 ± 229	657 ± 230
Legs *	Preterm (A)	159 ± 58	500 ± 155	736 ± 270	975 ± 354
	Term (B)	133 ± 73	451 ± 168	652 ± 300	830 ± 271
	Cross-Over (C)	129 ± 75	458 ± 133	602 ± 247	822 ± 409
	Breast-Fed (D)	98 ± 58	454 ± 183	654 ± 289	897 ± 254

* A > B ($p < .01$), C ($p < .001$), D ($p < .10$).

the preterm formula (Table 48.9). Thus, more rapid "catch-up" or "recovery" growth was paralleled by an increase in linear growth, lean and fat mass accretion, the latter primarily reflecting increased peripheral rather central fat mass accretion.

These data do not support the idea that adiposity is increased or altered in preterm infants fed a nutrient-enriched formula after hospital discharge when compared to infants fed a term formula or infants fed unfortified human milk. However, the data must be interpreted with caution. Body composition was measured using DEXA, which does not differentiate subcutaneous and visceral fat and subtle differences in visceral fat mass may have gone undetected. Insulin sensitivity/resistance was not determined and it is unclear if any relationship exists between insulin responses and dietary protein intake and/or central obesity in these high-risk infants.

48.7 Outstanding Issues

In their recent review on feeding preterm infants after hospital discharge the ESPGHAN Committee on Nutrition concluded that "close monitoring of growth" was needed to "enable the provision of adequate nutrition support" and that infants with a "subnormal weight for gestational age" who are a) breast-fed be supplemented with a human milk fortifier, b) formula-fed receive a post-discharge formula with "high contents of protein, minerals, trace elements and long-chain polyunsaturated fatty acid, at least until 40 weeks but possibly until 52 weeks post-conceptional age" [110].

Yet, many questions remain about the feeding of nutrient-enriched formulas to preterm infants after hospital discharge. The first relates to formula composition. What is the ideal composition of these formulas? Will one formulation meet the varying needs of a heterogeneous group of growth-retarded infants?

There are major differences in energy, protein, mineral and micronutrient content between formulas used in previous studies. While most studies showed a growth advantage, the magnitude of effect varied, the formulation associated with the most consistent growth advantage was that closest to a regular preterm formula [13, 14, 77].

Will one formulation meet the needs of boys and girls? To date, it is assumed that nutrient requirements are similar. However, preterm boys grow faster in-utero and accrete more lean mass than girls during the fist 2–3 months of life [86]. They are more susceptible to even marginal levels of intake and benefit more when fed nutrient-enriched formulas before [48, 111–113] and after hospital discharge [14, 78–80]. Future studies must be "powered" to detect differences between the sexes.

How long should these formulas be fed? In studies where the nutrient-enriched formula was fed to term or 2 months no advantage was noted. However, in studies where a nutrient-enriched formula was fed to 6, 9 and 12 months the most consistent advantage was noted. Whether 6 months is better than 9 or 12 months or vice-versa remains unclear.

Breast-fed infants appear likely to benefit with additional nutrient supplementation, however achieving this is problematic. Fortifiers could be used. Alternatively, a nutrient-enriched formula might also be fed once breastfeeding is fully established. Breastfeeding during the day supported by nutrient-enriched formula at night may not only improve growth but also prolong breastfeeding in these infants.

How often should infants be followed-up after hospital discharge? A "critical window of opportunity" appears to exist between discharge and 2–3 mca. Future studies must focus on this time frame, a time when infants should be frequently evaluated, using anthropometry and biochemical investigations as indicated and intake adjusted accordingly. Body composition and endocrine status might also be considered, at least under study conditions.

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49

Calcium and Phosphorus Homeostasis: Pathophysiology

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49.1 Introduction

Ninety-eight percent of the calcium and eighty percent of the phosphorus in the body are in the skeleton; these elements are also constituents of the intracellular and extracellular spaces. The metabolic homeostasis of calcium, phosphorus, and magnesium and mineralization of the skeleton are complex functions that require the intervention of various parameters; an adequate supply of nutrients; the development of the intestinal absorption process; and the effects of several hormones, such as parathyroid hormone, vitamin D, and calcitonin, as well as optimum renal and skeletal controls [1]. Bone formation requires protein and energy for collagen matrix synthesis, and an adequate intake of calcium and phosphorus is necessary for correct mineralization. During development, nutrients are transferred mainly across the placenta. It has been calculated that during the last trimester of gestation the daily accretion per kilogram of body weight represents around 120 mg of calcium and 70 mg of phosphorus. Therefore, at birth the whole-body content of a term infant represents approximately 30 grams of calcium and 16 grams of phosphorus. After birth, the use of the gastrointestinal tract to provide nutrients for growth causes a reduction in calcium availability for bone accretion promoting the occurrence of relative osteopenia in preterm infants and to a lesser extent in term infants during the first weeks of life. In addition to their roles in bone formation, calcium and phosphorus play important roles in many physiologic processes, such as transport across membranes, activation and inhibition of enzymes, intracellular regulation of metabolic pathways, secretion and action of hormones, blood coagulation, muscle contractility, and nerve conduction. The 20% of phosphorus not complexed within bone is present mainly as adenosine triphosphate, nucleic acids, and cell and organelle membranes.

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49.2 Calcium and Phosphorus Physiology

49.2.1 Calcium Physiology

49.2.1.1 Serum Calcium

Serum calcium is a fraction of total body calcium because only about 2% of calcium is present in extracellular fluid and soft tissues. Approximately 50% of total serum calcium is in ionized form at the normal serum protein concentration and represents the biologically active component of the total serum calcium concentration. Another 8% to 10% is complexed to organic and inorganic acids (e.g., citrate, lactate, bicarbonate, sulfate, and phosphate); together, the ionized and complexed calcium fractions represent the diffusible portion of circulating calcium. Approximately 40% of serum calcium is protein bound, primarily to albumin (80%) but also to globulins (20%) [2, 3]. Ionized calcium is the only physiologically active fraction.

The protein-bound calcium is not biologically active but provides a rapidly available reserve of calcium. Under normal circumstances the serum calcium concentration is tightly regulated by parathyroid hormone (PTH) and calcitriol (1,25-dihydroxy vitamin D_3; $1,25[OH]_2D_3$), which increase serum calcium (Fig. 49.1), and by calcitonin, which decreases serum calcium.

49.2.1.2 Placental Transport

During pregnancy there is an active calcium transfer from the mother to the fetus, reaching a peak of 120 to 150 mg/kg of fetal weight per day during the third trimester.

To meet the high demand for mineral requirements of the developing skeleton, the fetus maintains higher blood calcium and phosphorus levels than the ambient maternal levels. This process is the result of the active transport of calcium across the placenta by a calcium pump in the basal membrane that

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

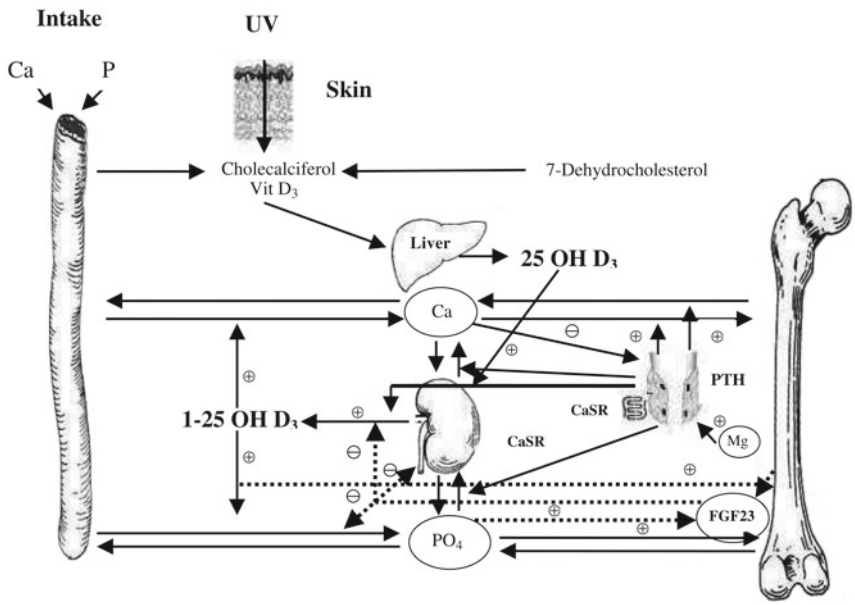

Fig. 49.1 Regulation of calcium and phosphate homeostasis. Parathyroid hormone (*PTH*) increases Ca release from bone, Ca resorption in the kidney, and $1,25(OH)_2D_3$ secretion from the kidney. PTH production is stimulated by low Ca and inhibited by low Mg and high $1,25(OH)_2D_3$. Vitamin D increases Ca release from bone and Ca and PO_4 absorption from the intestine. Vitamin D production is stimulated by high PTH and low PO_4. FGF23 production and release is stimulated by serum phosphate independent of PTH axis. FGF23 lowers phosphate concentration by inhibiting renal and intestinal phosphate transport as well as preventing activation of $1,25(OH)_2D_3$ which in turn controls levels of bone synthesis of FGF23. *CaSR* calcium-stimulating response, *UV* ultraviolet light

maintains a gradient of maternal to fetal calcium of 1:1.4. PTH has no effect on placental calcium transport.

By contrast, the main regulator of fetal ionized calcium appears to be the parathyroid hormone-related protein (PTHrP) produced by the placenta. PTHrP(1-141) or its midmolecule fragment, PTHrP(67-86), is the active form contrary to PTH (1-34) or PTHrP(1.34), suggesting that PTHrP stimulates receptor(s) distinct from the PTH/PTHrP receptor [4].

The role of vitamin D in fetal physiology is not well understood. Cord concentrations of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D correlate significantly with those found in the maternal circulation, suggesting that the vitamin D pool of the fetus depends entirely on that of the mother. Fetomaternal relationships of 1,25-dihydroxyvitamin D concentrations are still debated.

Nevertheless, it has been demontrated that the placenta is able to synthethise and metabolize 1,25-dihydroxyvitamin D through the activity of 25-hydroxyvitamin D-1α-hydroxylase and 1,25-dihydroxyvitamin D-24 hydroxylase, two key enzymes for vitamin D metabolism [5]. In addition, it has been suggested that methylation of the vitamin D-24-hydroxylase gene plays an important role in maximizing active vitamin D bioavailability at the fetomaternal interface [6] and that active transplacental calcium and phosphate transfers are at least partly dependent on insulin-like growth factor-1 (IGF-1), itself a stimulating factor of fetal and placental 1,25-dihydroxyvitamin D synthesis.

Bone mass of the newborn may be related to the vitamin D status of the mother and studies of particular populations show that infants of mothers severely deficient in vitamin D may be born with rickets and can suffer fractures in the neonatal period [7, 8].

49.2.1.3 Intestinal Absorption

Calcium absorption is the main determinant of its retention; consequently, it has a significant impact on bone mineral content. Calcium absorption occurs in the small intestine by both active and passive processes. Vitamin D is essential for the active absorption of calcium, which involves carriers such as calcium-binding proteins.

After birth calcium absorption limits the rate of bone mineralization for growth although the absorption rate is higher than that during all other periods of life. The absorption of calcium occurs in the small intestine by two main routes – either through or between cells. Movement through the cell takes place by active transport dependent on vitamin D bound to a cytosolic calcium-binding protein (calbindin D_{9k}). The major role of vitamin D in transcellular calcium transport involves the biosynthesis of calcium-binding protein. Passive calcium transport is driven by chemical gradients that represent movement of calcium among the cells (paracellular transport). In addition to vitamin D status, various other factors affect calcium absorption [2, 9]. Ionization of calcium compounds, which requires an acid pH, occurs in the stomach and is a prerequisite for absorption. Therefore, low availability could be the result of an insoluble fraction of calcium intake or the precipitation of calcium in the gut. Calcium chloride, citrate, and carbonate have higher solubilities than calcium phosphate, which should be avoided in formulas. By contrast, the higher solubility of organic calcium, such as that found in calcium gluconate or glycerophosphate, improves its absorption. Fat intake and LCSFA (long-chain saturated fat acids) intake may also influence calcium absorption through the formation of calcium soap. It has been showed that the free palmitate content in the gastrointestinal tract after

the hydrolysis of triglyceride may impair calcium absorption. The higher bioavailability of calcium in human milk would be partly due to palmitate, which is predominantly esterified at the glycerol 2 positions (Sn-2), but also to the human milk bile salt-stimulated lipase that is not specific to the Sn-1 and Sn-3 positions. The improvement of calcium absorption with the use of medium-chain triglycerides is probably the result of the reduction of total LCSFA content in formula. At present, with the use of a well absorbed fat combination (±85% fat absorption), the influence of calcium soap formation and the B-palmitate content formula could be relatively minimal in clinical practice. A relatively low gastrointestinal pH content or the lactose and casein content of the formula may also have an additional positive effect on calcium absorption. In light of the considerable requirements of preterm infants, all of these factors probably play a significant role in the amount of calcium retained and deposited in the skeleton.

Medications also interfere with calcium absorption; for example, glucocorticoids inhibit intestinal transfer. Some anticonvulsants can also inhibit calcium absorption either directly (phenytoin) or indirectly through interference with vitamin D metabolism (phenobarbital and phenytoin). In newborn infants, a significant amount of calcium is secreted in the intestinal lumen through digestive fluid. With the use of stable isotopes, endogenous fecal calcium excretion and true calcium absorption may be evaluated. Considering that fecal calcium excretion may represent about 15 mg/kg per day in preterm infants, the true calcium absorption rate is significantly higher than the apparent rate measured by conventional metabolic balances.

Numerous metabolic balance studies have been performed in preterm infants fed human milk or a formula (Fig. 49.2) [1, 10, 11] to evaluate apparent calcium absorption. In preterm infants fed human milk, calcium absorption ranges from 60% to 70% depending on the calcium intake,

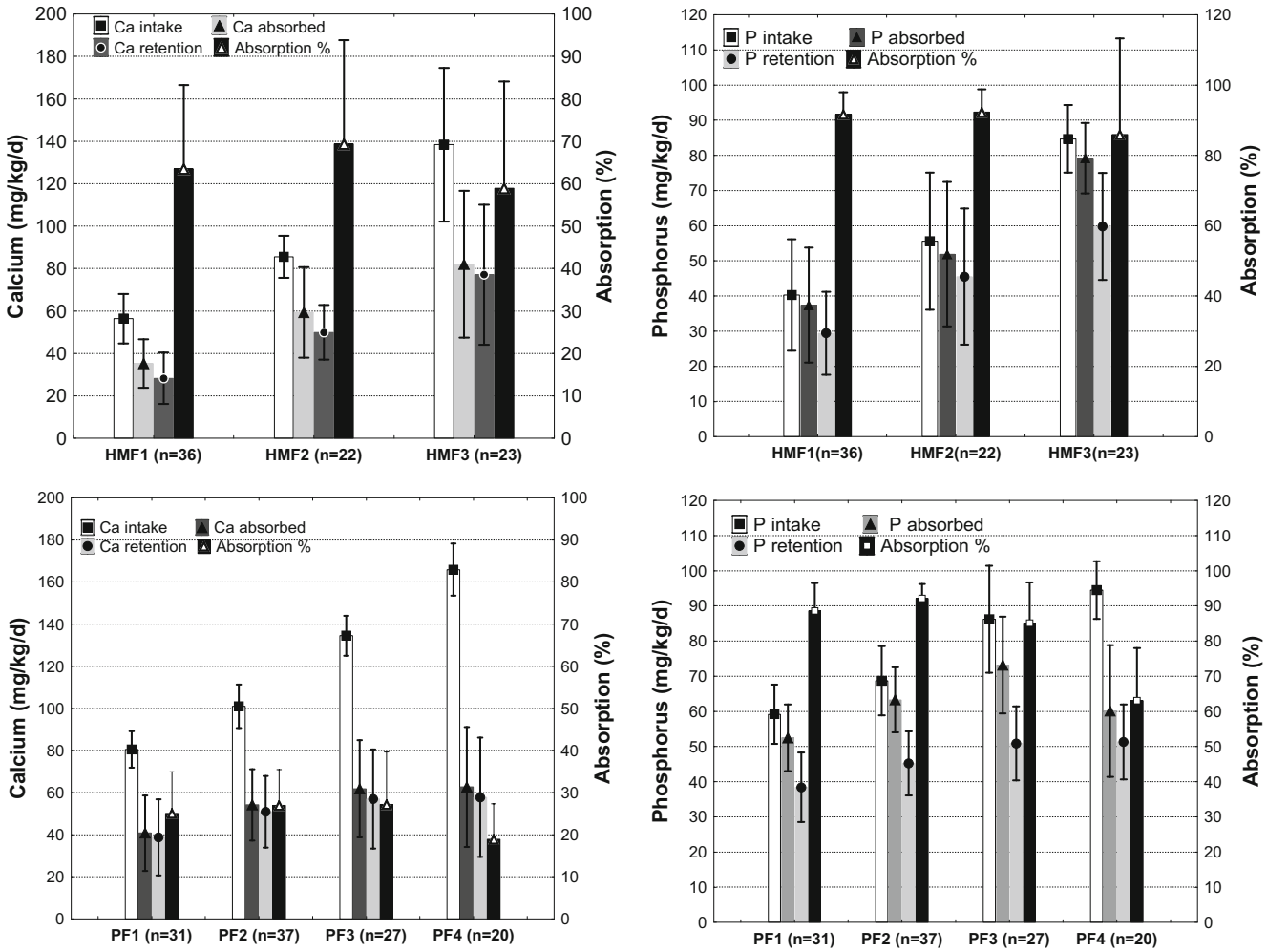

Fig. 49.2 Calcium and phosphorus absorption and retention in preterm infants fed human milk (without or with a human milk fortifier) and preterm formulas. In human milk groups, calcium and phosphorus absorption and retention are related to intake, in contrast to formula groups, in which a plateau is reached rapidly due to a decrease in net absorption (%). *HMF* human milk fortifier, *PF* preterm formula

whereas calcium retention is related to the phosphorus supply. Supplementation of human milk with phosphorus alone normalizes calciuria and allows calcium retention to reach about 35 mg/kg per day. When calcium and phosphorus are provided together or as human milk fortifiers, calcium retention and absorption rates parallel that of human milk, reaching 60 mg/kg per day. The use of new human milk fortifiers containing highly soluble calcium glycerophosphate improves calcium retention to 90 mg/kg per day (Fig. 49.2) [1]. In formula-fed infants, the percentage of net calcium absorption is less than that with human milk, ranging from 35% to 60%. Most of the difference probably results from the various factors affecting calcium solubility and absorption. At present, the use of a preterm formula with a high mineral content does not necessarily improve mineral retention [10]. Indeed, due to the poor solubility of calcium salts, especially calcium phosphate, the calcium content measured in formulas may be significantly lower than the claimed value, and an additional loss due to precipitation may occur before feeding [10]. In metabolic studies, the actual amount of calcium provided by feeding needs to be measured. As shown in Fig. 49.2, calcium retention limited to 90 mg/kg per day could presently be expected in preterm infants fed a preterm formula with a highly soluble calcium content. Nevertheless, those values are still relatively far from the reference values calculated during the last trimester of gestation (120 to 130 mg/kg per day), which is still considered the target mineral accretion rate for infants with very low birth weight (VLBW).

49.2.1.4 Renal Excretion

Under normal circumstances, calcium status is maintained by a balance between its intestinal absorption and renal excretion. Urinary excretion is the result of glomerular filtration followed by the sum of the processes of tubular reabsorption and secretion. Approximately 70% of the filtered load of calcium is reabsorbed in the proximal tubule and 20% in the thick ascending limb of Henle's loop in association with Na^+ reabsorption. Although it is responsible for only 5% to 10% of Ca^{2+} reabsorption, the major regulation of Ca^{2+} reabsorption occurs in the distal convoluted tubule by a mechanism independent of Na^+ reabsorption but regulated by PTH and $1,25(OH)_2D_3$. Calcium reabsorption is increased by both PTH and $1,25(OH)_2D_3$. It is also regulated by ionized calcium concentration, phosphate concentration, and acid-base status and increases with Ca^{2+} depletion and alkalosis but decreases with hypercalcemia, phosphate depletion, and acidosis. The effects of diuretics on renal calcium excretion vary considerably. Furosemide markedly increases renal calcium losses and is a risk factor for neonatal nephrocalcinosis. Thiazides increase renal tubular calcium reabsorption, thereby reducing calciuria [12].

The ability of the kidney to dispose of excess calcium represents a major homeostatic mechanism. Under normal circumstancs, nearly all filtered calcium (98%) is reabsorbed in the renal tubule. However, preterm and term infants differ from the adult in three main aspects: (1) Renal function is far from being completely developed, (2) Mineral requirements for growth are very high and (3) Renal calcium load results solely in the difference between net absorption and net bone and soft tissue retention. Considering that calcium soft tissue retention is negligible, renal calcium load is highly dependent on bone calcium deposition associated with phosphorus in the form of hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ containing a molar calcium:phosphorus ratio of 1.67 (2.15 wt/wt). Therefore, the main determinant of the urinary loss of calcium in preterm and term neonates is relative to phosphorus depletion, which has been illustrated in balance studies performed in growing preterm infants fed human milk. When human milk is provided exclusively, there is an increase in the urinary excretion of calcium associated with very low urinary phosphate excretion. In contrast, when human milk is supplemented with phosphate, significant phosphaturia appears at the same time that calciuria decreases to a minimum level. Therefore, hypercalciuria can be explained by a relative phosphate depletion that cannot meet the phosphate demand necessary for skeletal mineralization (Fig. 49.3).

49.2.1.5 Requirement

For preterm infants, recommendations are based on fetal accretion rates. In 1985 the American Academy of Pediatrics recommended that formulas contain 140 to 160 mg of calcium per 100 kcal [13]. More recently, the Life Sciences Research Office recommended a calcium content of 123 to 185 mg/100 kcal for preterm infant formula, and an international expert panel suggested a similar value of 90 to 180 mg/100 kcal [14]. However, the ESPGHAN (European Society for Pediatric Gastroenterology, Hepatology, and Nutrition) committee of nutrition considers that those calcium needs are based on data obtained in infants fed preterm formulas with low calcium bioavailability. It suggests that after birth, bone metabolism is designed to accelerate bone turnover, reducing calcium requirements and that the relative osteopenia observed in preterm infants could be considered at least partially physiologic. As a calcium retention level ranging from 60 to 90 mg/kg/day assures appropriate mineralization and decreases the risk of fracture in VLBW infants, the ESPGHAN CoN recommended the use of highly bioavailable calcium salts providing a calcium content of 110-130 mg/100 kcal [15].

49.2.2 Phosphorus Physiology

Unlike calcium, phosphorus remains in the soft tissues, mainly in the form of phosphate esters, and in extracellular fluid in the form of inorganic phosphate ions. It represents approximately 15% of the whole-body content. Given its

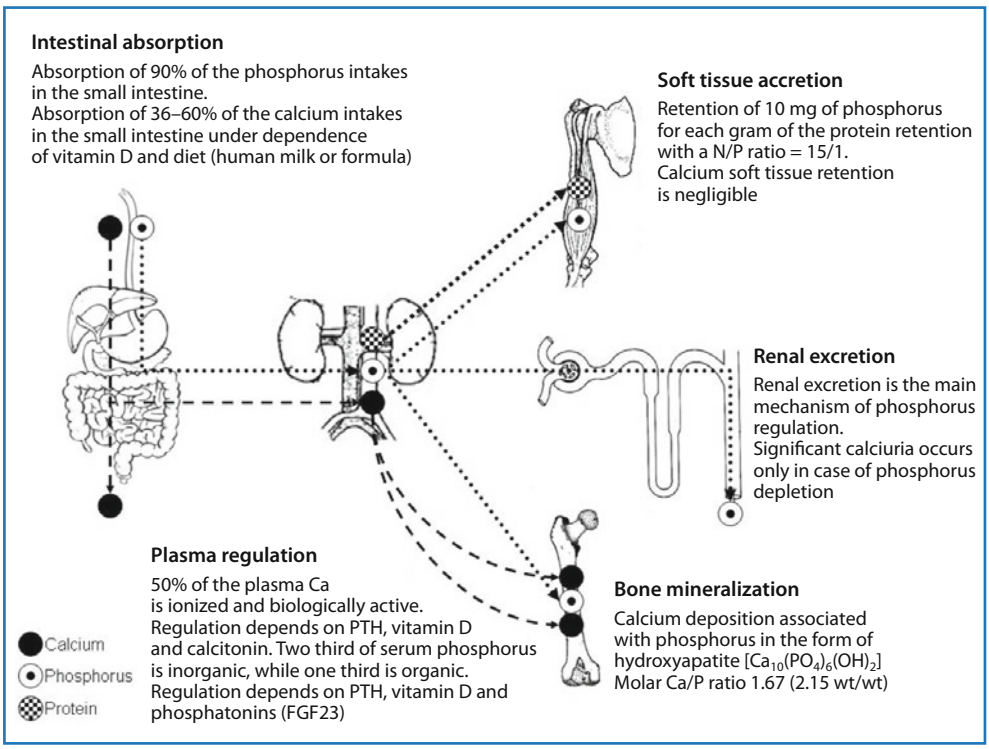

Fig. 49.3 Metabolism of calcium and phosphorus in term infants with feeding

widespread distribution, phosphorus plays a critical role in many biological processes, including energy metabolism, membrane composition, nucleotide structure, cellular signaling and bone mineralization. It is, therefore, not surprising that a deficiency of phosphorus results in clinical disease, including muscle weakness, impaired leukocyte function, and abnormal bone metabolization.

49.2.2.1 Serum Phosphorus

In serum about two thirds of phosphorus is organic (lipid phosphorus and phosphoric ester phosphorus) and one third inorganic. In routine clinical practice only inorganic phosphorus is measured. About 85% of the inorganic phosphorus is ionized, circulating as monohydrogen or dihydrogen phosphate; 5% is complexed with sodium, magnesium, or calcium; and 10% is protein bound [2].

Because so many phosphorus species are present, depending on pH and other factors, the serum concentration is conventionally expressed as the mass of the elemental phosphorus (mmol/L or mg/dL). In contrast to calcium, the serum phosphorus concentration varies widely depending mainly on intake and renal excretion but is also influenced by age, gender, pH, and a variety of hormones. At birth, the mean serum phosphorus concentration is relatively low (2.6 mmol/L, or 6.2 mg/dL) but thereafter rises rapidly to reach 3.4 mmol/L, or 8.1 mg/dL, owing to both endogenous phosphorus release and low renal excretion. Serum phosphorus subsequently varies. The diet partially determines the serum phosphorus content: it is higher in formula-fed than breast-fed infants. Serum phosphorus is inversely related to serum calcium concentration. After the neonatal period, the serum phosphorus concentration progressively decreases to 2.1 mmol/L, or 5 mg/dL, at the age of 1 to 2 years; 1.8 mmol/L, or 4.4 mg/dL, in middle childhood; and 1.5 mmol/L, or 3.5 mg/dL, at the end of adolescence [12].

49.2.2.2 Placental Transport

During pregnancy there is transfer of phosphorus from the mother to the fetus that reaches a peak of 60 to 75 mg/kg per day during the third trimester; 75% is retained for bone mineralization, and 25% is retained in other tissues. The transplacental transport of phosphorus is an active process against a concentration gradient and is sodium dependent. Both $1,25(OH)_2D_3$ and fetal PTH may be involved in the regulation of placental phosphorus transfer.

49.2.2.3 Intestinal Absorption

Intestinal phosphorus absorption takes place primarily in the duodenum, jejunum, and to a lesser extent in the ileum and colon. It occurs via two mechanisms: an active, sodium-dependent transcellular process localized to the mucosal surface

and passive diffusion through the paracellular pathway. It depends on both the absolute amount of dietary phosphorus and the relative concentrations of calcium and phosphorus (an excessive amount of either can decrease the absorption of the other). Vitamin D may stimulate active phosphorus absorption, although it is largely independent of vitamin D intake. The efficiency of this absorption is high (close to 90% of intake) regardless of the type of milk given [1, 2]. However, the use of poorly soluble phosphate salt such as calcium triphosphate in formulas is associated with a significant reduction in phosphorus absorption [1]. In contrast to calcium, only a small amount of phosphate is secreted in the intestinal lumen through digestive fluid.

49.2.2.4 Renal Excretion

The kidney contributes to a positive phosphate balance during growth by the reabsorption of a relatively high fraction of filtered inorganic phosphate (99% in newborns, 95% in infants fed human milk, and 80% in adults).

Preterm infants have an increased fractional excretion of phosphate and are at a greater risk of developing signs and symptoms of phosphate deficiency. The bulk of filtered phosphate is reabsorbed in the proximal tubule via a sodium-dependent transporter, the Na^+-phosphate cotransporter. The age-related decrease in phosphate reabsorption observed after the weaning period appears to be correlated with the smaller phosphate needs of adult and older animals than those of growing animals and is associated with a rapid downregulation of Na^+-phosphate cotransporter-2 mRNA and protein in the brush border membrane [16]. The kidney is the major determinant of the plasma phosphate concentration. Filtered load depends on the plasma phosphorus level and glomerular filtration rate (GFR).

Tubular reabsorption is an active and saturable process that gives rise to a maximum rate of tubular reabsorption (T_m). There is a plasma minimum threshold below which phosphorus reabsorption is almost complete and urinary excretion close to zero and a maximum threshold above which all tubular reabsorptive systems are saturated, so each additional increment in filtered load is associated with a parallel increment in excretion [2]. In preterm infants, the minimum and maximum threshold levels are 1.75 mmol/L (5.4 mg/dL) and 2.45 mmol/L (7.6 mg/dL), respectively (Fig. 49.4).

In the intermediate zone, there is a functional relationship between GFR and T_m, known as the glomerulotubular balance, in which a change in GFR is compensated for by a change in T_m in order to regulate phosphorus excretion. The total body phosphate metabolism is tightly regulated by three hormones, namely PTH, $1,25(OH)_2$ vitamin D and fibroblast growth factor 23 (FGF23) [17]. The level of PTH activity in plasma is an important physiologic regulator of phosphorus excretion. PTH inhibits phosphorus reabsorption, but its activity appears to be limited to the intermediate zone between the minimum and maximum plasma threshold levels. In contrast, $1,25(OH)_2$ vitamin D synthesis, stimulated by a decrease in plasma phosphorus concentration, has an indirect effect on phosphorus reabsorption by its effect on mineral absorption and mobilization from bone, resulting in an increase in plasma calcium concentration and the suppression of PTH release. During early postnatal life the phosphate response to PTH is blunted, whereas PTH increases tubular calcium reabsorption.

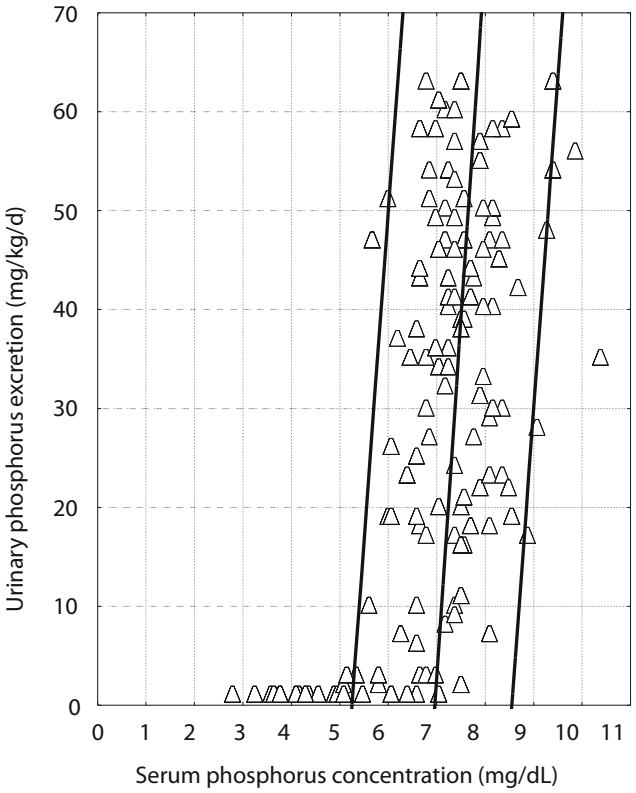

Fig. 49.4 Relationship between urinary excretion of phosphorus and serum phosphate level (n = 198) in preterm infants. Regression lines represent the minimum, mean, and maximum plasma phosphate concentration thresholds for tubular reabsorption of phosphate. Points to the left of the minimum threshold were considered hypophosphatemia associated with phosphorus depletion. Points to the right of the maximum threshold were considered hyperphosphatemia due to low glomerular filtration rates and relative phosphorus overload

Together, these actions result in the retention of both calcium and phosphate in infants, which is favorable for growth. The role of newly discovered phosphatonin peptides such as FGF23 in the regulation of phosphorus excretion during the early life remain to be established. FGF23 principally functions as a phosphaturic factor and counter-regulatory hormone for $1,25(OH)_2$ vitamin D production [17].

Absorbed phosphate enters the extracellular phosphate pool, which is in equilibrium with bone and soft tissue. In adults with neutral phosphorus balance, the amount of phos-

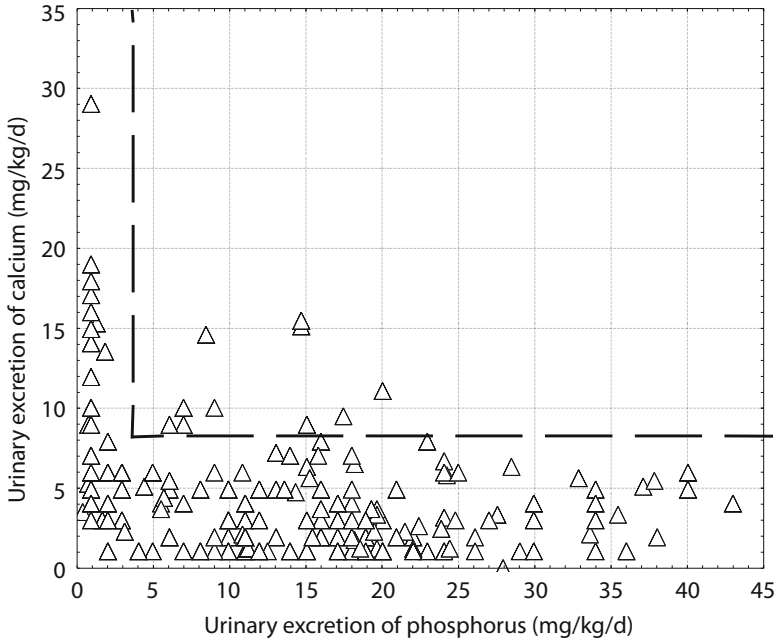

Fig. 49.5 Relationship between urinary excretion of calcium and urinary excretion of phosphorus in preterm infants (n = 198). Hypercalciuria (>8 mg/kg/day) is related to low phosphate excretion (<3 mg/kg/day). Conversely, urinary excretion of calcium below 8 mg/kg/day is usually observed in preterm infants with phosphorus excretion over 4 mg/kg/day

phorus excreted by the kidney is equal to the net amount absorbed by the intestine; in growing infants, it is less than the net amount absorbed due to the deposition of phosphorus in soft tissues and bone.

In growing infants, phosphorus will preferentially go to soft tissue with a weight-to-weight nitrogen:phosphorus ratio of 15:1 and to bone with a weight-to-weight calcium:phosphorus ratio of 2.15:1. The residual phosphorus constitutes the renal phosphorus load influencing plasma concentration and urinary excretion. In the face of a limited total phosphorus supply, bone mineral accretion may be limited, leading to significant calcium excretion associated with very low urinary excretion of phosphorus (Fig. 49.5). This particular situation is illustrated in Fig. 49.3, showing calcium and phosphorus metabolism in term infants.

49.2.2.5 Requirement

For preterm infants the recommendations are based on fetal accretion rates. The 1985 American Academy of Pediatrics recommended 95 to 108 mg of phosphorus per 100 kcal for preterm formulas [13], whereas more recently, the Life Sciences Research Office and the International Expert Panel recommended similar values, with a calcium-to-phosphorus ratio maintained over 1.7:1 but less than 2.0:1 [14]. Considering a nitrogen retention ranging from 350 to 450 mg/kg/day and a calcium retention from 60 to 90 mg/kg/day. The ESPGHAN CoN recommended an adequate intake of 65 to 90 mg/kg/day of a highly absorbable source of phosphate (90%) with a Ca to P ratio between 1.5 and 2.0 [15].

49.3 Hormonal Regulation

49.3.1 Parathyroid Hormone

PTH is synthesized as a larger (115-amino acid) precursor (prepro-PTH) but is stored and secreted mainly as an 84-amino acid peptide, with the 1-34,N-terminal portion conferring bioactivity. PTH regulates large calcium fluxes across bone, kidneys, and intestine (Fig. 49.1). PTH increases serum calcium concentrations directly by increasing bone resorption and renal calcium reabsorption and indirectly by increasing renal synthesis of $1,25(OH)_2D_3$, thereby increasing intestinal calcium absorption. PTH also lowers serum phosphorus concentrations through its phosphaturic action on the renal proximal tubule. This action minimizes the possibly adverse effect of hyperphosphatemia related to bone resorption on calcium homeostasis.

49.3.1.1 Regulation

Serum calcium concentration regulates PTH secretion; high concentrations inhibit the secretion of PTH and low concentrations stimulate it. Low or falling serum calcium concentrations act within seconds to stimulate PTH secretion, initiated by means of a calcium-sensing receptor (CaSR) on the surfaces of parathyroid cells. Slower regulation of PTH secretion occurs over a period of hours as a result of cellular changes in PTH messenger RNA (mRNA). Vitamin D and its metabolites 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, acting

through vitamin D receptors, decrease the level of PTH mRNA and hypocalcemia increase it. The slowest regulation of PTH secretion occurs over days or even months and reflects changes in the growth of the parathyroid glands. Metabolites of vitamin D directly inhibit the mass of parathyroid cells; hypocalcemia stimulates the growth of parathyroid cells independent of the contrary action of vitamin D metabolites. In the kidney, the calcium-sensing receptor (CaSR) mediates the direct inhibition of the reabsorption of divalent cations in the thick cortical ascending limb of Henle's loop [18].

49.3.1.2 Fetal Parathyroid Function

Human parathyroid glands are functionally active as early as 12 weeks of gestation. However, fetal parathyroid glands are functionally suppressed by high intrauterine calcium concentrations, and PTH levels in cord blood are frequently below the detection limit. PTH does not appear to cross the placenta in either direction, and the role of PTH in maternal-fetal calcium transport is limited. In contrast, PTHrP acts during fetal life to promote calcium transfer, high blood calcium concentration, bone development, and matrix mineralization.

Maternal hyperparathyroidism results in maternal hypercalcemia, which leads to fetal hypercalcemia and suppression of fetal and neonatal parathyroid glands. Conversely, untreated maternal hypoparathyroidism leads to maternal hypocalcemia, fetal hypocalcemia, and secondary fetal and neonatal hyperparathyroidism.

49.3.1.3 Neonatal Parathyroid Function

After birth, with the abrupt termination of the maternal calcium supply, serum calcium in the newborn decreases and serum PTH increases correspondingly. Both term and preterm infants may show a PTH response to falling serum calcium. Nevertheless, plasma PTH levels are relatively low in the neonatal period and are minimally responsive to hypocalcemia during the fist 2 to 3 days of life leading to transient neonatal hypocalcemia. Infants with VLBW have a decreased PTH surge compared with full-term infants. Infants of diabetic mothers may have impaired PTH production during the beginning days of life. Infants with birth asphyxia may also have decreased PTH responses to hypocalcemia.

49.3.1.4 Parathyroid Hormone Reference Values

In full-term newborns, serum PTH concentrations tend to be low in cord blood but increase within the first 48 hours of life in response to the decrease in serum calcium. In preterm infants the serum immunoreactive, intact PTH (1-84) concentrations increase immediately after birth, indicating that the secretion of the hormone responds physiologically to the hypocalcemic stimulus. This increase in immunoreactive PTH concentration could be blunted when premature infants receive calcium by infusion, with the calcium load buffering the postnatal depression of serum calcium. By day 10, the serum concentrations of intact PTH return to euparathyroid values (Table 49.1) [7]. The multiplication factor for serum PTH from picograms per deciliter to picomoles per liter (pg/dL to pmol/L) is 0.11.

Table 49.1 Evolution of intact PTH (1–84), carboxy terminal PTH, and vitamin D binding protein concentrations in 15 preterm infants

	PTH(1–84) (pmol/L)	cPTH (pmol/L)	DBP (μmol/L)
Cord Serum	11 ± 3	48 ± 8	4.43 ± 0.37
Day 1	66 ± 11*	125 ± 15*	4.40 ± 0.34
Day 2	87 ± 11*	168 ± 5*	4.96 ± 0.23
Day 5	67 ± 9*	152 ± 16*	6.21 ± 0.26*
Day 10	23 ± 4	69 ± 6	6.03 ± 0.30*
Day 30	38 ± 7	80 ± 11	5.16 ± 0.23

* Significantly different from cord serum, $P < .05$.
PTH parathyroid hormone, *cPTH* carboxy terminal PTH, *DBP* vitamin D binding protein.
From [7], with permission.

49.3.2 Vitamin D

49.3.2.1 Synthesis and Metabolism

Vitamin D is synthesized endogenously in the skin (cholecalciferol or vitamin D_3) after sunshine exposure or is absorbed from dietary sources as either vitamin D_3 (from animal sources) or vitamin D_2 (ergocalciferol [from vegetable sources]). Regardless of its origin, vitamin D_2 and D_3 are transported bound to the vitamin D binding protein to the liver, where it is hydroxylated at carbon 25 to form vitamin D_3 (25[OH]D_3, or calcidiol), the most abundant vitamin D metabolite. The circulating concentrations of 25[OH]D_3 provide a useful index of vitamin D status (reflecting both dietary intake and sunshine exposure). Subsequently in the kidney, 25[OH]D_3 is further hydroxylated at carbon 1 to form the final active metabolite, 1,25$(OH)_2D_3$, or calcitriol. This last transformation is tightly regulated and is the rate-limiting step in vitamin D metabolism. 1,25$(OH)_2D_3$ can also be synthesized by various cells as well as by the placenta during pregnancy. However, this local production of 1,25$(OH)_2D_3$ is not associated with calcium homeostasis but could contribute to regulating cell growth.

49.3.2.2 Effects

Normal vitamin D status is necessary to maintain calcium and phosphorus homeostasis. The effects of 1,25$(OH)_2D_3$

(calcitriol) on target tissues are initiated by its binding to a steroid receptor (vitamin D receptor) distributed in numerous tissues, leading to the synthesis of a variety of proteins. Therefore, $1,25(OH)_2D_3$ acts on the small intestine, increasing the absorption of calcium and phosphorus by the synthesis of calcium-binding (calbindin-D) proteins; on bone, mobilizing calcium and phosphorus by increasing the number of osteoclasts; and on the kidney, increasing calcium reabsorption in the distal nephron segments by increasing the expression of the epithelial calcium influx channel. There is a natural polymorphism in the genotype of the vitamin D receptor that contributes to skeletal metabolism in early childhood and to the genetic determinant of the peak bone mass.

49.3.2.3 Regulation of Secretion

In contrast to hepatic 25-hydroxylation, renal 1-hydroxylation, which leads to the active metabolite, appears to be tightly regulated. The main factors increasing the synthesis of calcitriol via stimulation of renal $25(OH)D_3$-1α-hydroxylase are PTH, PTHrP, hypocalcemia, hypophosphatemia, and other hormonal factors, such as insulin-like growth factor-1, estrogen, prolactin, and growth hormone. The production of calcitriol is inhibited by elevated serum levels of calcium and phosphorus, but also by the newly discovered phosphatonin peptides implicated in phosphorus homeostasis. Thus, an increase in FGF23 production suppresses $1,25(OH)_2$ vitamin D via inhibition of 25-hydroxyvitamin D-1α-hydroxylase and stimulation of 24-hydroxylase inactivating $1,25(OH)_2$ vitamin D in the proximal tubule of the kidney [17].

49.3.2.4 Fetal Vitamin D Function

Serum 25-hydroxyvitamin D concentration depends on vitamin D intake and production. The production of vitamin D is influenced by geographic location, season and latitude, and skin pigmentation. Vitamin D deficiency is very common during pregnancy, not only in areas with a prolonged winter season. In Belgium, vitamin D deficiency, < 50 and 80 nmol/L, represent respectively 80 and 90% of the pregnant women [19].

Cord concentrations of the major vitamin D metabolites are consistently lower than those measured in the mother's serum. Placental vein 25-hydroxyvitamin D concentrations correlate significantly with those found in the maternal circulation, suggesting that calcidiol easily diffuses across the placental barrier and that the vitamin D pool of the fetus depends entirely on that of the mother. The fetomaternal relations of 1,25-dihydroxyvitamin D concentrations are more complex, most of the 1,25-dihydroxyvitamin D in fetal plasma is due to fetal kidney activity, as suggested by studies in fetal plasma from infants with renal agenesis. Actually, the precise role of the placental 1,25-dihydroxyvitamin D activity in the mineral homeostasis during the fetal life remains to be evaluated.

Bone mass of the newborn may be related to the vitamin D status of the mother [20]. Comparison of the results of dual-energy X-ray absorptiometry from different countries shows that infant whole-body bone mineral content values are lower in countries in which milk products are not supplemented with vitamin D than in those where milk products are supplemented. In contrast, vitamin D supplementation of malnourished mothers results in improved growth of the fetus and child in terms of both birth weight and subsequent linear growth during infancy.

Therefore, during pregnancy a daily vitamin D supplement of at least 400 IU/day (10 μg/day) need to be administered, whereas for at-risk populations an increased supply to 1000 IU/day [25 μg/day]) should be given at least during the last 3 months of pregnancy.

49.3.2.5 Neonatal Vitamin D: Function and Recommendations

Plasma $25(OH)\ D_3$ concentration is a useful vitamin D biomarker reflecting vitamin D supply and usage over a period of time. Unfortunately, the cut-off used for serum $25(OH)\ D_3$ concentrations varies among researchers, leading to various prevalence of vitamin D deficiency and/or insufficiency. Nevertheless, several surveys show a high rate of poor maternal vitamin D status throughout the world, particularly in countries without vitamin D supplementation, with poor sun exposure, extensive clothing, or with deeply pigmented skin. Thus, the rate of cord blood vitamin D severe insufficiency (< 20 nmol/L; < 8.3 ng/mL) may reach up to 70% in European populations [19, 20].

The concentrations of vitamin D are low in human milk (20 to 60 IU/L) and lower than that in regular formulas (400 to 600 IU/L). In breast-fed infants not receiving vitamin D supplements, vitamin D stores could be depleted within 8 weeks of delivery, suggesting the need for vitamin D supplementation [21]. A minimum daily intake of 400 IU (10 μg/day) of vitamin D is recommended by the AAP for all infants beginning soon after birth, including those who are exclusively breastfed [22]. Moreover, in breastfed full-term infants born to mothers who are vitamin D deficient, maternal supplementation with vitamin D could be required to increase the human milk vitamin D concentration and in turn improve infant vitamin D status. In addition, vitamin D is metabolized in the liver, and anticonvulsants such as phenobarbital and diphenylhydantoin increase its hepatic catabolism and requirements.

In preterm infants, as a result of the decrease in serum calcium during the first days of age, the postnatal surge in PTH induces increased synthesis of $1,25(OH)_2D_3$ during the first days day of life. Provision of 1000 IU of vitamin D_3 increases the 25-hydroxyvitamin D pool of the newborn at

birth, followed by rapid renal synthesis of 1,25-dihydroxyvitamin D during the early postnatal days. In very low birth weight infants (<1500 g), immaturity of the vitamin D activation pathway, either alone or in combination with other abnormalities, particularly transient hypoparathyroidism, hypercalcitoninemia, and end-organ resistance to hormonal effects, may promote late neonatal hypocalcemia. However, after 28 weeks of gestation, activation of vitamin D is operative as early as 24 hours after birth. Vitamin D supplementation just after birth improves its nutritional status, as evidenced by rises in both plasma 1,25- and 25-hydroxyvitamin D concentrations.

In countries with high vitamin D status, plasma 25-hydroxyvitamin D remained normal for 6 months while infants received around 400 IU/day (<10 μg/day). By contrast, in Europe and other countries where dairy products are not enriched with vitamin D, sunshine exposure is restricted [7], and mean cord concentrations of 25-hydroxyvitamin D are low, the administration of vitamin D (from 1000 IU/day, or 25 μg/day) results in a rapid increase in circulating concentrations of total 1,25-dihydroxyvitamin D by 5 days of age, promoting calcium absorption and mineral accretion rates during the neonatal period. Therefore, vitamin D recommendations may differ significantly between North American (400 IU/day, 10 μg/day) and European countries (800 to 1000 IU/day, 10 to 25 μg/day) [15].

49.3.2.6 Reference Values

New informations on the additional role of vitamin D on glucose homeostasis, immune system, cardiovascular disease, and cancer has resulted in defining vitamin D deficiency in adults as a 25-OH-D concentration <50 nmol/L and vitamin D insufficiency as a 25-OH-D concentration of 50 to 80 nmol/L. At the present time, however, consensus has not been reached with regard to the concentration of 25-OH-D to define vitamin D insufficiency for infants and children [23]. Universal units of measure for 25-OH-D and 1,25-OH_2-D are nmol/L. Conversion to ng/mL is made by dividing the value expressed in nmol/L by 2.496. Thus, 80 nmol/L becomes 32 ng/mL.

49.3.3 Calcitonin

Calcitonin (CT) is a 32-amino acid peptide secreted by the thyroidal C cells. In addition, a group of peptide hormones, the "calcitonin family", including the calcitonin gene-related protein (CGRP), with structural similarities to calcitonin are also produced by several tissues. Although calcitonin has often been considered to be a vestigial hormone with no certain role in mammalian calcium and bone homeostasis, recent studies indicate that calcitonin has important roles in calcium and bone homeostasis that have not been previously recognized. It has been established that the major action of CT is the inhibition of bone resorption, and osteoclasts [24]. The regulation of calcitonin is opposite to that of PTH. Mediated by the CaSR, an increase in ionized calcium stimulates calcitonin secretion, whereas a decrease in ionized calcium leads to a fall in calcitonin. Thus, the CaSR has a dual physiological response to regulate PTH and calcitonin that in the long-term will influence the maintenance of skeletal mineral content [25].

At birth, serum calcitonin concentrations are higher in cord blood than maternal blood, and they increase further in the first 24 hours of life. The physiologic importance of the postnatal increase of calcitonin is unclear and it is uncertain whether calcitonin plays any specific role in calcium homeostasis and skeletal metabolism during the fetal and neonatal period [25]. In contrast, CT has an important physiological role during lactation protecting the maternal skeleton against excessive resorption and attendant fragility.

49.3.4 FGF23 and Phosphatonin Peptides

"Phosphatonins" represent a number of peptides which have been recently identified as a result of the study of various diseases associated with hypophosphatemia: fibroblast growth factor 23 [FGF23], secreted frizzled-related protein 4 [sFRP-4], fibroblast growth factor 7 [FGF7] and matrix extracellular phosphoglycoprotein [MEPE] [17, 26, 27]. FGF23 is predominantly produced by osteocytes in bone, and its principal actions are to inhibit sodium-dependent phosphate reabsorption and 1α-hydroxylase activity in the proximal tubule of the kidney, leading to phosphaturia and suppression of circulating 1,25$(OH)_2$D levels. Evidence that FGF23 is the principle phosphaturic factor comes from a variety of observations [27].

Fibroblast growth factor 23 regulates serum phosphate levels within a narrow range despite wide fluctuation in dietary intake via a series of classic negative endocrine feedback loops involving 1,25-dihydroxyvitamin D, parathyroid hormone, urinary phosphate excretion, and dietary phosphorus absorption. A sustained increase in dietary phosphorus intake stimulates FGF23 secretion that increases phosphaturia. At the same time, FGF23 inhibits renal synthesis of 1,25D. By contrast, a sustained reduction in phosphorus intake lowers FGF23 secretion, which enhances tubular phosphorus avidity and increases 1,25D production. A down regulation of FGF-23 could promote the relative hyperphosphatemia and the relative increase in 1,25$(OH)_2$ vitamin D promoting bone mineralization during early rapid growth in the neonatal period [17]. The targeting of FGF23 to the kidney is mediated by a coreceptor (klotho) that enhances the binding affinity of FGF23 to the widely expressed FGF receptor 1c [17].

Phosphatonins have been implicated in the pathophysiology of disorders of phosphate homeostasis such as Tumor-induced osteomalacia (TIO), X-linked hypophosphatemic rickets (XLH), autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemia (ARHP),

tumoral calcinosis and renal failure [27]. However, the role of newly discovered phosphatonin peptides, such as FGF 23 in the regulation of phosphorus excretion during the early life, remain to be established.

49.4 Clinical Conditions Associated with Calcium Disturbances

49.4.1 Neonatal Hypocalcemia

Serum total and ionized calcium concentrations are relatively high at birth but decrease sharply during the first hours of life to reach a nadir at 24 hours and increase progressively thereafter up to the end of the first week of life (Table 49.2). Sudden changes in the distribution of calcium between ionized and bound fractions may cause symptoms of hypocalcemia even in children with functioning hormonal mechanisms for the regulation of the ionized calcium concentration (Table 49.3). Increases in the extracellular fluid concentration of anions such as phosphate, citrate, or bicarbonate increase the proportion of bound calcium and decrease ionized calcium. Alkalosis increases the affinity of albumin for calcium and thereby decreases the concentration of ionized calcium. In contrast, acidosis increases the ionized calcium concentration by decreasing the binding of calcium to albumin. Although it is conventional to measure the total serum calcium concentration, more physiologically relevant information is obtained by direct measurement of the ionized calcium concentration. This measurement is particularly important when evaluating patients who have abnormal circulating proteins and after blood transfusion for the correction of acidosis and hyperventilation. For example, total serum calcium decreases approximately 1 mg/dL, or 0.25 mmol/L, for each 1 g/dL of decrease in serum albumin, without any change in ionized calcium.

Neonates at the greatest risk for symptomatic or asymptomatic neonatal hypocalcemia, such as the infants of diabetic mothers or preterm or asphyxiated neonates, are frequently sick for a multitude of reasons, and the contribution of neonatal hypocalcemia to signs related to their primary illness can be easily obscured. From a clinical viewpoint, because Ca^{2+} concentrations are maintained within narrow ranges under normal circumstances, the potential risk for disturbances of physiologic function increases as the Ca^{2+} concentration

Table 49.2 Evolution of serum calcium concentrations (mmol/L*) during the first 10 days of life in term and preterm infants

	Term		Preterm	
Age	Mean	95% CI	Mean	95% CI
Birth (cord blood)	2.55	2.25-2.85	2.24	1.58-2.90
24 h	2.25	1.95-2.55	1.94	1.64-2.24
48 h	2.39	2.14-2.64	1.85	1.47-2.23
120 h	2.46	2.25-2.68	2.22	1.84-2.60
240 h	2.48	2.26-2.69	2.45	2.45-2.89

*Conversion to mg/dL: mmol/L × 0.2495.

Table 49.3 Causes of neonatal calcium disorders

Hypocalcemia	Hypercalcemia
Early Hypocalcemia (1-4 days of age)	*Iatrogenic*
Prematurity	Calcium salts, vitamin A
Maternal diabetes	Hypophosphatemia (prematurity)
Perinatal stress/asphyxia	Hypervitaminosis D
Intrauterine growth restriction	Thiazide diuretics
Maternal anticonvulsants	
	Disorders of Parathyroid Function
Late Hypocalcemia (5-10 days of age)	Maternal hypocalcemia, hypoparathyroidism
Hyperphosphatemia (high phosphate load, advanced renal insufficiency)	Mutation of the parathyroid hormone-related protein receptor
Hypomagnesemia	Jansen metaphyseal chondrodysplasia
Vitamin D deficiency	Calcium-sensing receptor defects
PTH resistance (transient neonatal pseudohypoparathyroidism)	Familial hypocalciuric hypercalcemia
Hypoparathyroidism	Neonatal severe hyperparathyroidism
Primary: parathyroid agenesis, 22q11 deletion, parathyroid hormone gene mutation	*Idiopathic Infantile Hypercalcemia*
Secondary: maternal hyperparathyroidism	*Hyperprostaglandin E Syndrome*
Calcium-sensing receptor defects; autosomal dominant hypocalcemic hypercalciuria	*Severe Infantile Hypophosphatasia*
Acquired or inherited disorders of vitamin D metabolism	*Other Causes*
Neonatal hypocalcemia associated with skeletal dysplasia	Congenital carbohydrate malabsorption
Other causes (alkalosis, citrated blood transfusions, phototherapy, viral gastroenteritis, lipid infusions)	Distal renal tubular acidosis
	Tumor-related hypercalcemia
	Congenital hypothyroidism
	Williams syndrome
	Subcutaneous fat necrosis
	Blue diaper syndrome

decreases. A useful approach to the classification of neonatal hypocalcemia is by time of onset. The early and late forms of hypocalcemia have different causes and occur in different clinical settings.

49.4.1.1 Early Hypocalcemia - Term Infants

Early neonatal hypocalcemia occurs during the first 4 days of life and represents an exaggeration of the normal fall in serum calcium concentration that occurs during the first 24 to 48 hours of life. At birth there is an interruption of maternal calcium supply, and the serum calcium concentration in infants is maintained by either the increased calcium flux from bone or sufficient exogenous calcium intake. Because intestinal calcium absorption is correlated with intake and dietary calcium is usually low on the first day of life, the serum calcium concentration decreases on the first day of life [3].

Normal serum concentrations for Ca^{2+} in full-term newborns reach a nadir at about 24 hours of age (1.10 to 1.36 mmol/L, or 4.4 to 5.4 mg/dL) and rise slowly thereafter. Neonatal hypocalcemia could be reasonnably defined as a serum total calcium below 2 mmol/L or a an ionized calcium of less than 0.9 to 1.1 mmol/L.

Early neonatal hypocalcemia apparently results from the abrupt interruption of the placental supply and the low intake provided by oral and parenteral nutrition and also by the slow release of PTH by immature parathyroid glands or the inadequate responsiveness of the renal tubular cells to PTH.

By contrast, exaggerated rise in calcitonin secretion may not play a contributory role in premature infants. In infants with VLBW the high renal sodium excretion probably aggravates calciuric losses, and relative end-organ resistance to $1,25(OH)_2D_3$ may exist additionally to an $25(OH)D_3$ deficiency. Hypocalcemia is temporary, and the serum calcium concentration gradually reverts to normal after 1 to 3 days. Factors contributing to serum calcium normalization include increased calcium intake with feedings, increased renal phosphorus excretion, and improved parathyroid function. Calcium supplementation may hasten the restorative process.

49.4.1.2 Early Hypocalcemia - Preterm Infants

The frequency of hypocalcemia varies inversely with birthweight and gestational age. In preterm infants, the postnatal decrease in the serum calcium level typically occurs more rapidly than it does in term infants, the magnitude of the depression being inversely proportional to gestation. Before routine calcium supplementation and the use of parenteral nutrition from the first day of age, many LBW infants and nearly all those with extremely low birth weight (ELBW) exhibited total calcium levels of less than 7.0 mg/dL by day 2. However, the fall in Ca^{2+} is not proportional to that in total calcium concentration, and the ratio of ionized to total calcium is higher in these infants. The reason for the maintenance of Ca^{2+} is uncertain but is probably related to low serum protein concentration and pH associated with prematurity. The sparing effect of Ca^{2+} may partially explain the frequent lack of signs in preterm infants with low total calcium levels. In preterm infants the reference values for ionized calcium are available only for large, moderately premature infants who show values very similar to those for full-term infants. These cutoff values might not apply to smaller infants, given insufficient physiologic data on ionized calcium. At present, the traditional cutoff point, a total calcium content of less than 1.75 mmol/L (7.0 mg/dL) and an ionized calcium of less than 0.9 to 1.1 mmol/L, remains reasonable in VLBW infants.

49.4.1.3 Perinatal Asphyxia

In asphyxiated infants, the following factors may contribute to early hypocalcemia: a decreased calcium intake due to delayed feedings and an increased endogenous phosphorus load resulting from the reduction of the glomerular filtration rate. Hyperphosphatemia may induce relative PTH resistance and a stimulation of FGF23 secretion. Theoretically, the correction of acidosis with alkali may further aggravate hypocalcemia by inducing decreased calcium flux from bone to the extracellular fluid and by lowering the ionized calcium concentration.

49.4.1.4 Maternal Conditions and Treatments

Maternal Diabetes

Infants of diabetic mothers (IDMs) demonstrate an exaggerated postnatal drop in circulating calcium levels when compared with controls of gestational age. Prematurity and birth asphyxia are frequently associated problems that independently increase the risk for hypocalcemia. In IDMs, hypocalcemia appears to be related to hypomagnesemia, the maternal form of which is caused by urinary magnesium losses with diabetes and leads to fetal magnesium deficiency and secondary functional hypoparathyroidism in the fetus and newborn. Hypocalcemia in IDMs is also correlated with the severity of maternal diabetes, which is classified by White's criteria. The natural history is usually similar to that of early neonatal hypocalcemia in preterm infants, but hypocalcemia sometimes persists for several additional days. Improved metabolic control for pregnant diabetic women has markedly diminished the occurrence and severity of early neonatal hypocalcemia in IDMs. The incidence of hypocalcemia is also increased in the infants of gestational diabetic mothers (IGDMs), and the role of hypomagnesemia has recently been demonstrated [28].

Maternal hyperparathyroidism with hypercalcemia, maternal chronic renal failure with secondary hyperparathy-

roidism, mothers with abnormal vitamin D homeostasis and mothers receiving anticonvulsants are additional factors increasing risk for neonatal hypocalcemia (Table 49.3).

49.4.1.5 Late Hypocalcemia

Hypocalcemia is conventionally defined as late when it occurs after the first 4 days of life. Late neonatal hypocalcemia usually develops at about 1 week of age (Table 49.3) and more frequently in term than preterm infants and is not correlated with maternal diabetes, birth trauma, or asphyxia. In some instances the clinical distinction between early and late hypocalcemia may not be clear.

Phosphate Loading

Hypocalcemia induced by an elevated phosphorus supply usually occurs at the end of the first week of life. Late-onset hypocalcemia is considered to be a manifestation of relative resistance of the immature kidney to PTH. In these infants the renal tubular cells are unable to respond appropriately to PTH, leading to renal retention of phosphorus and hypocalcemia. These biochemical features strongly resemble those of pseudo-hypoparathyroidism [3]. The normally low neonatal glomerular filtration rate may also play a role in limiting the ability to excrete the phosphorus load.

Late hypocalcemia was frequently observed in infants fed cow's milk or evaporated milk because of their high phosphorus content. With the introduction of adapted infant formulas, late hypocalcemia, though not abolished, has become uncommon. However, even with current formulas the formula-fed infants have lower serum ionized calcium and higher serum phosphorus in the first week of life than those of breastfed infants.

These differences correlate with the absolute phosphorus amount but not with the different calcium:phosphorus ratios in formulas. The phosphate load increases calcium bone deposition, leading to hypocalcemia. The normal response to hypocalcemia is an increase in PTH secretion, inducing an increase in both the urinary excretion of phosphate and tubular resorption of calcium. The pathogenesis of this "transient hypoparathyroidism" in late neonatal hypocalcemia is poorly understood. The inadequate secretion of PTH, immaturity of PTH receptors, or transient change in the threshold of CaSR may play an important role [29]. In these infants, serum calcium levels frequently increase when they are given human milk, lower-phosphate formulas, and calcium supplements. After several days to weeks, serum PTH usually increases and the infants are able to tolerate a higher dietary phosphate load. Some of these infants have a persistent or recurrent inability to mount an adequate PTH response to a hypocalcemic challenge and may have a form of congenital hypoparathyroidism.

Hypocalcemia Resulting from Vitamin D Disorder

Maternal vitamin D deficiency is the major risk for neonatal vitamin D deficiency presenting as hypocalcemia. Vitamin D deficiency is unusual in countries where it is common practice to supplement the diet with vitamin D dairy products and other foods. However, it occurs in women in whom both sunlight exposure and the dietary intake of vitamin D are inadequate and high incidence has been reported in most of the european countries [19]. Therefore, if daily vitamin D supplementation during the whole pregnancy can be undertaken, the amount given should be at least 400 IU/day (10 μg/day). In countries where dairy products are not supplemented with vitamin D and sunshine exposure is low, 1000 IU/day (25 μg/day) should be given during the last 3 months of pregnancy [7].

Breastfed infants of strictly vegetarian mothers are also susceptible to vitamin D deficiency and early-onset hypocalcemic rickets.

Hypomagnesemia

Neonatal hypocalcemia usually accompanies hypomagnesemia because magnesium deficiency inhibits the secretion of PTH and reduces responsiveness to its action. Depression of the serum magnesium levels in newborns is mainly due to transient hypomagnesemia or exceptionally to primary hypomagnesemia with secondary hypocalcemia [3].

49.4.1.6 Other Causes of Neonatal Hypocalcemia

Bicarbonate therapy, as well as any form of metabolic or respiratory alkalosis, decreases ionized calcium levels and bone resorption of calcium. Transfusion and plasmapheresis with citrated blood can form nonionized calcium complexes, thus decreasing Ca^{2+}. Furosemide and xanthine therapy promotes calciuresis as well as nephrolithiasis. Phototherapy appears to be an additional possible cause of neonatal hypocalcemia, although the mechanism is still uncertain. Lipid infusions may increase serum free fatty acid levels, which form insoluble complexes with calcium. Most of these effects are transient, and cessation of therapy is associated with a return to normal serum calcium levels.

49.4.1.7 Clinical Manifestations of Hypocalcemia

The clinical manifestations of neonatal hypocalcemia in infants may be easily confused with other neonatal disorders (e.g., hypoglycemia, sepsis, meningitis, asphyxia, intracranial bleeding, narcotic withdrawal). The neonate with hypocalcemia may be asymptomatic; the less mature the infant, the more subtle and varied the clinical manifestations. In the neonatal period the main clinical signs of hypocalcemia are

jitteriness (increased neuromuscular irritability and activity) and generalized convulsions, although focal seizures have also been reported. Infants may also be lethargic, eat poorly, vomit, and have abdominal distention. The degree of irritability does not appear to correlate with serum calcium values. Furthermore, hypocalcemia may be asymptomatic. Therefore, suspicion of hypocalcemia should be confirmed by the measurement of total serum calcium and Ca^{2+}.

The diagnostic workup for hypocalcemia includes a history, physical examination, and relevant investigations. In clinical practice, the diagnosis of hypocalcemia is based on the determination of ionized or total calcium. Serum magnesium should also be measured because hypomagnesemia may coexist and cause identical signs. The measurement of calcium-regulating hormones is not routinely recommended unless hypocalcemia is prolonged, refractory, or recurrent. Assays of calciotropic hormones and 25(OH)D may be useful in the diagnosis of uncommon causes of neonatal hypocalcemia, such as primary hypoparathyroidism, malabsorption, and disorders of vitamin D metabolism. If DiGeorge syndrome is suspected chest X-ray examination for a thymic silhouette is indicated and molecular genetic studies may be needed to confirm a microdeletion of chromosome 22q11.2.

49.4.1.8 Treatment

Early Neonatal Hypocalcemia

In asymptomatic newborns, treatment generally is indicated when the total serum calcium concentration is less than 1.5 mol/L (6 mg/dL) in the preterm infant and less than 1.75 mol/L (7 mg/dL) in the term infant. Calcium supplementation can be given either by the intravenous or oral route. Depending on the clinical status of the infant, treatment of newborns who have acute or symptomatic hypocalcemia is accomplished best by the intravenous infusion of calcium salts; 10% calcium gluconate (9.3 mg/mL of elemental calcium, maximum 2 mL/kg over 15 min followed by 75mg/kg per day up to normal range) is used most commonly. Effective oral therapy involves adding calcium glucobionate or calcium carbonate to human milk or a low phosphate formula (calcium to phosphate ratio of 2:1).

Additional therapy depends on the cause of hypocalcemia. Infants who have hypoparathyroidism require calcitriol in addition to calcium supplementation to restore and maintain eucalcemia. Infants who have vitamin D deficiency will benefit from vitamin D supplementation. If magnesium concentrations are low, treatment with intravenous or intramuscular magnesium sulfate is essential for correction as a 50% solution of magnesium sulfate in a dose of 0.1 to 0.2 mL/kg.

Complications of intravenous calcium therapy include extravasation into soft tissues (with calcium deposition and sometimes cutaneous necrosis) and bradycardia. Because of the many potential risks, arterial infusions of calcium in high concentrations should be avoided. However, parenteral nutrition solutions containing standard mineral (including calcium) content can be safely infused through appropriately positioned umbilical venous or arterial catheters. The direct administration of calcium preparations with bicarbonate or phosphate solution results in precipitation and must be avoided. The duration of supplemental calcium therapy varies with the course of hypocalcemia. As few as 2 or 3 days of therapy are usually required.

Late Neonatal Hypocalcemia

There is usually little debate regarding the treatment of late hypocalcemia. Because serum calcium is not routinely measured after the first few days of life, late hypocalcemia is usually symptomatic when diagnosed and the requirement for calcium therapy may be prolonged in the case of hypocalcemia caused by malabsorption or hypoparathyroidism. In phosphorus-induced hypocalcemia, a low-phosphorus formula (or human milk) and oral calcium supplementation are indicated to decrease phosphorus absorption and increase calcium absorption. In hypomagnesemia, the magnesium deficiency usually has to be corrected before hypocalcemia can be treated successfully

Hypoparathyroidism requires therapy with vitamin D or one of its metabolites; $1,25(OH)_2D_3$ (or 1 α-hydroxyvitamin D_3, a synthetic analogue that undergoes hepatic 25-hydroxylation) has the advantage f a shorter half-life, and treatment may be more easily tailored to the individual patient. Attention should be directed toward a number of concomitant treatments. Because thiazide diuretics can increase renal calcium reabsorption, the inadvertent institution or discontinuation of these drugs may increase or decrease, respectively, the plasma calcium level. In contrast, furosemide and other loop diuretics can increase the renal clearance of calcium and depress serum calcium levels. The administration of glucocorticoids antagonizes the action of vitamin D (and the analogues) and may also precipitate hypocalcemia. The development of hypomagnesemia may also interfere with the effectiveness of treatment with calcium and vitamin D [2].

49.4.1.9 Prevention of Neonatal Hypocalcemia

The most effective prevention of neonatal hypocalcemia includes the prevention of prematurity and birth asphyxia, the judicious use of bicarbonate therapy, and mechanical ventilation to avoid secondary metabolic alkalosis. Whereas, the pharmacologic prevention of neonatal hypocalcemia, especially in VLBW infants, is based on the prophylactic use of calcium salts, phosphorus salts, and vitamin D supplementation from the first day of life, with a regular survey of serum concentrations and urinary excretion.

49.4.2 Neonatal Hypercalcemia

Hypercalcemia is defined as a pathologic elevation in plasma ionized Ca^{2+} concentration greater than 1.35 mmol/L (5.4 mg/dL) with or without a simultaneous elevation in total calcium concentration of greater than 2.75 mmol/L (11.0 mg/dL) as total calcium is more related to serum albumin concentration [2, 18]. Neonatal hypercalcemia (Table 49.3) is relatively uncommon, but it needs to be recognized because it can result in significant morbidity or mortality. The clinical symptoms of sustained hypercalcemia are not specific. Infants with mild increases in serum calcium (2.75 to 3.25 mmol/L, or 11 to 13 mg/dL) often fail to manifest specific symptoms of hypercalcemia. Nonspecific signs and symptoms such as anorexia, vomiting, and constipation (but rarely diarrhea) may occur with moderate to severe hypercalcemia. Seizures, bradycardia, or arterial hypertension is very exceptional. On physical examination infants may appear dehydrated, lethargic, and hypotonic. Those with chronic hypercalcemia may present with failure to thrive as the principal source of physical distress. Renal function is generally impaired, and polyuria and hypercalciuria are observed. However, renal complications such as nephrocalcinosis, nephrolithiasis, and hematuria may be the earliest clinical manifestations of hypercalcemia.

49.4.2.1 Iatrogenic Hypercalcemia

Hypercalcemia may result from increased intestinal or renal calcium absorption, increased bone turnover or iatrogenic causes. Iatrogenic hypercalcemia is the most common type of hypercalcemia in the newborn and should be considered before starting extensive investigation of rare syndromes. It may result from excessive intravenous calcium administration during total parenteral nutrition or exchange transfusion but also of unbalanced calcium to phosphorus ratio in the oral or parenteral diet. Moderate hypercalcemia may also be the result of phosphorus deficiency in premature infants receiving unbalanced calcium and phosphorus regimens in oral and parenteral nutrition. In this situation, hypercalcemia is accompanied by hypophosphoremia. Infants with VLBW who are fed mineral-unsupplemented human milk may develop hypophosphatemic hypercalcemia. The low phosphorus concentration in human milk causes phosphorus deficiency, leading to an increased calcium concentration and hypercalciuria. Hypophosphatemia stimulates renal synthesis of calcitriol, which activates the intestinal absorption and skeletal resorption of calcium and phosphorus. The absorbed phosphorus is preferentially oriented for soft tissue formation, whereas the remaining phosphorus is insufficient to allow calcium deposition. Phosphorus supplementation and the use of human milk fortifiers can prevent hypophosphatemia and hypercalcemia. Similar situations have been reported in infants on parenteral nutrition, providing an unbalanced calcium:phosphorus ratio. Additionally, thiazide diuretics reduce renal calcium excretion and may represent a contributing factor. Other causes of iatrogenic hypercalcemia are the use of extracorporeal membrane oxygenation, which can cause transient hypercalcemia in up to 30% of infants, and vitamin D intoxication from the administration of excessive vitamin D supplements. Vitamin A toxicity increasing bone resorption is rare and can cause severe hypercalcemia.

49.4.3 Neonatal Hyperparathyroidism

Neonatal hyperparathyroidism frequently results in severe hypercalcemia. It may be hereditary primary hyperparathyroidism or be secondary to maternal hypocalcemia and is summarized in Table 49.3 with other more unusual causes of hypercalcemia.

49.4.3.1 Primary Hyperparathyroidism

Hereditary primary hyperparathyroidism manifested in neonates is associated with inactivating mutation of CaR. The serverity of hypercalcemia is related to the extent of CaR mutation, moderate with heterozygous mutation in patients with familial hypocalciuric hypercalcemia. It is an autosomal-dominant trait with a high degree of penetrance. More severe hypercalcemia occurs in neonatal hyperparathyroidism with homozygous inactivating germline mutations of the CaR gene [30].

49.4.3.2 Secondary Hyperparathyroidism

In many cases of secondary hyperparathyroidism, a thorough investigation of the infant's mother will disclose previously known but poorly treated hypoparathyroidism, pseudohypoparathyroidism, or clinically unsuspected hypocalcemia, as seen in mothers with renal tubular acidosis that had induced severe secondary hyperparathyroidism in the developing fetus during pregnancy. The clinical presentation is variable and may depend on the severity of maternal hypo- calcemia. Secondary hyperparathyroidism and neonatal hypercalcemia are transient conditions with a good prognosis provided that supportive measures are instituted.

49.4.3.3 Clinical Manifestations

Most infants are asymptomatic when diagnosed. Those with mildly elevated levels of calcium often fail to manifest specific symptoms of hypercalcemia. Infants with chronic hypercalcemia may present with failure to thrive as the principal source of physical distress.

There are nonspecific signs and symptoms such as anorexia, vomiting, and constipation (but rarely diarrhea); polyuria may occur with moderate to severe hypercalcemia. In severe hypercalcemia infants are often dehydrated, lethargic,

and hypotonic. Alternatively, they may present with seizures. Clinically, these infants can have bradycardia, a short QT interval, and hypertension. However, renal complications such as nephrocalcinosis, nephrolithiasis, and hematuria may be the earliest clinical manifestations of hyper- calcemia. Otherwise, the physical examination is usually normal except for the infants with subcutaneous fat necrosis, Williams syndrome, Jansen metaphyseal chondrodysplasia, and hypophosphatasia.

In clinical practice the practical approach is first to exclude the possibility of iatrogenic hypercalcemia. Secondly, a maternal history of calcium-phosphorus disease or excessive vitamin D intake during pregnancy should be investigated. Thirdly, the signs of clinical syndromes associated with hypercalcemia, such as blue diapers, fat necrosis, and elfin facies, should be sought. Finally, the initial laboratory evaluation should include serum calcium, phosphorus, alkaline phosphatase, PTH, the urinary calcium:creatinine ratio, and tubular reabsorption of phosphorus. In most cases, these tests allow the differentiation of hypercalcemia caused by parathyroid disorders from nonparathyroid conditions. In hyperparathyroidism the serum phosphorus concentration is low; renal tubular phosphorus reabsorption is decreased, usually to less than 85%; and the serum PTH concentration is elevated. Finally, additional tests may be performed: a serum $25(OH)D_3$ determination may be useful when an excess of vitamin D is suspected; long-bone X-ray films identify demineralization, osteolytic lesions, or both (hyperparathyroidism) or osteosclerotic lesions (occasionally with vitamin D excess); measurements of serum and urinary calcium in parents allow a diagnosis of familial hypocalciuric hypercalcemia; and renal sonography detects nephrocalcinosis.

49.4.3.4 Treatment

The treatment of neonatal hypercalcemia depends on the severity of the presentation. Conservative management is appropriate in the case of mild hypercalcemia in a preterm infant resulting from an inappropriate mineral supply, hypoalbuminemia, or chronic acidosis, with special emphasis on the phosphorus supply when hypercalcemia is associated with hypophosphatemia. Hypercalcemia seen in newborns exposed to maternal hypocalcemia is usually mild and transient, and treatment consists of no more than supplying the appropriate amounts of calcium and phosphorus in the milk.

Infants with moderate to severe hypercalcemia need more aggressive treatment. The initial steps are not specific: (1) Discontinue oral and intravenous calcium and vitamin D supplementation and dietary restriction; (2) increase the urinary excretion of calcium by maximizing glomerular filtration with the administration of intravenous fluids, which consist of standard saline at about twice the maintenance requirements; and (3) encourage calcium excretion with furosemide after rehydration but with particular attention to maintaining electrolyte homeostasis.

More specific therapy comprises the use of glucocorticoids, calcitonin, bisphosphonate, dialysis, and total parathyroidectomy. Glucocorticoids (2 mg/kg of prednisone) decrease intestinal calcium absorption, decrease bone resorption, and increase renal excretion. They may be useful during a short period, mainly in cases of an excess of vitamin D, but are relatively ineffective in cases of hyperparathyroidism. Calcitonin (4 to 6 IU/kg subcutaneously every 6 hours) reduces the serum calcium concentration, but its effectiveness declines after a few days. Bisphosphonate therapy is limited in newborn infants. However, pamidronate (0.5 to 2.0 mg/kg) has been used in the treatment of subcutaneous fat necrosis and could be an ideal agent to stabilize cases of neonatal severe hyperparathyroidism, as recently suggested. A calcimimetic drug that reduces PTH secretion, recently approved by the FDA for chronic secondary hyperparathyroidism in dialyzed patients, could be of major interest in primary hyperparathyroidism but needs to be evaluated during infancy [29]. Dialysis could be prescribed with a low-calcium dialysate (1.25 mmol/L) in the face of severe and unremitting hypercalcemia. Total parathyroidectomy with partial autotransplantation could be a rescue treatment in the severe form of neonatal severe hyperparathyroidism. In the chronic phase, dietary restriction with the use of a special formula without vitamin D supplementation is the mainstay of treatment. If the dietary regimen is insufficient, corticosteroids can be used with caution. Cellulose phosphate binders have been occasionally used in children, but there is limited experience in neonates and they may contain unwanted free phosphate.

49.4.4 Nephrocalcinosis in Preterm Infants

Nephrocalcinosis is defined as ultrasound evidence of bright reflections with or without acoustic shadowing, small flecks across to completely echodense pyramids which are reproducible in both the longitudinal and transverse direction, found within the cortex or medulla. Initially, nephrocalcinosis was attributed to long-term furosemide therapy. It is now known to be multifactorial and has been associated with low gestational age and birth weight; severe respiratory disease, transient renal failure; imbalance intakes of calcium and phosphate; long duration of total parenteral nutrition; as well as pro-calciuric medications such as furosemide, corticosteroids, aminoglycosides, and xanthines. In addition, in analogy to the pathogenesis of stone formation, nephrocalcinosis is considered to result from a spontaneous or therapy-induced imbalance between promoters (e.g., calcium, oxalate, uric acid, ascorbic acid) and inhibitors (e.g., citrate, magnesium) of crystallization in urine. The prevalence of nephrocalcinosis varies between studies from 7% to 41% in recent reports [31].

The short- and long-term evolution of nephrocalcinosis has not been clearly defined. Ultrasonographic abnormalities that develop during the first months of life disappear in the majority of patients within months to years. Earlier studies had raised

concerns regarding tubular function in affected patients. This has not clearly been substantiated in more recent larger studies where an unfavorable effect on renal function is seen in a small number of children [31]. Nevertheless, premature children with or without nephrocalcinosis when compared with non-premature children had lower kidney size and borderline blood pressure. In addition, children with intra- or extrauterine growth retardation had an impaired GFR compared to children with appropriate pre- and postnatal growth [32].

In summary, nephrocalcinosis is a high incidence disease in VLBW and ELBW infants but associated with a good renal outcome and a high resolution rate. However, it has been highlighted that there could be long-term sequelae in tubular dysfunction and hypertension. This warrants close monitoring in ex-premature babies.

49.5 Disorders of Phosphate Homeostasis

49.5.1 Neonatal Hypophosphatemia

In adults, moderate hypophosphatemia is defined as a serum phosphorus concentration between 3.0 and 1 mg dL^{-1}, and is usually asymptomatic. Severe hypophosphatemia is defined as serum inorganic phosphorus concentration below 1.0 mg dL^{-1}. In children, serum phosphorus concentrations below 5 mg dL^{-1} are often considered abnormal. This level corresponds to the threshold level of tubular resorption capacity and potential increase in urinary calcium excretion (Fig. 49.5). Hypophosphatemia may be caused by decreased intestinal absorption of phosphate, increased urine losses of phosphate, and an endogenous shift of inorganic phosphorus from extracellular to intracellular fluid compartments. It has also been reported in various rare hereditary diseases associated or not with neonatal hyperparathyroidism (Table 49.4). Early hypophosphatemia was observed in IUGR. Within the 1st week of life, a significant correlation between preeclampsia and 1st week inorganic phosphate deficiency was related in VLBW babies, whereas, the occurrence of hypercalciuria and low urinary phosphate was also observed in small-for gestational age (SGA) infants. These studies suggest that infants with extrinsic IUGR are probably insufficiently provided with phosphorus *in utero* and hence postnatally may suffer early-onset inorganic phosphate depletion from prenatal origin. This mineral imbalance has been delineated in newborns with chronic fetal hypoxia and malnutrition leading to disproportionate or type II IUGR. In those infants, hypophosphatemia as well as platelet count and high nucleated red blood cell count were closely correlated with the severity of IUGR in SGA type II.

A high incidence (i.e., 28%) of hypophosphoremia associated with a double mortality rate related to myocardial dysfunction is reported in adult intensive care patients. A short course of phosphotherapy improved the left ventricular function rapidly in inorganic phosphate depleted adults [33].

Table 49.4 Causes of neonatal phosphorus disorders

Hypophosphatemia	Hyperphosphatemia
Endocrine	*Endocrine*
Hyperparathyroidism	Hypoparathyroidism
VitaminD deficiency or resistance	Hyperthyroidism
Renal losses	Growth hormone excess
Congenital tubular disorders	Vitamin D toxicity
Secondary tubular disorders	*Renal*
Diuretics	Renal failure
Volume expanders	Volume depletion
Hypercalciuria	*Increased load*
Glucosuria	Enteral, Cow milk feeding
Hypomagnesemia	Rectal enema
Gastrointestinal	Parenteral
Decrease of intake	Blood transfusion
Decrease of absorption	
Calcium salt	
Malabsorption	
Antacid	
Gene mutation	
Others	
Metabolic acidosis	
Respiratory alkalosis	
Gram-negative sepsis	
Refeeding syndrome	
Hypercalcemia	
Carbonic anhydrase inhibitors	
Dopamine	

Therefore it is tempting to speculate that early-onset inorganic phosphate depletion could be an etiologic factor in myocardial dysfunction, leading postnatally to left heart failure and ensuing pulmonary hemorrhage in the most severely growth retarded VLBW-infants.

During the first weeks of life, hypophosphatemia related to low phosphorus intake occurs in low birth weight babies who were fed soy formulas and more frequently exclusive unsupplemented human milk [34]. With human milk addition of 5 to 10 mg/100 mL of inorganic phosphate equivalent corrects plasma concentration, reduces calcium excretion and improves calcium retention. In formula fed infants, similar hypophosphatemia is also observed in relation to an inadequate calcium phosphorus ratio resulting either from a relative phosphorus malabsorption or a relatively higher calcium absorption rate. In that situation, absorbed phosphorus is lower than that required for deposition in soft tissues and bone according to nitrogen retention and calcium absorption.

An inadequate calcium to phosphorus ratio is also observed in preterm infants with persistent metabolic acidosis or prolonged respiratory distress syndrome. In that situation, the acid-base disturbance, increased bicarbonate or hypercapnia reduced phosphorus tubular reabsorption inducing a relative hypophosphatemia and hypercalciemia associated with a concomitant urinary excretion of phosphorus and calcium increasing the risk of nephrocalcinosis.

Given its widespread distribution and critical role in vital cellular processes, it is not surprising that a deficiency of

phosphorus results in various clinical symptoms including hypotension and decreased stroke volume, impaired diaphragm contractility, dyspnea and respiratory failure, paresthesia, weakness, confusion, disorientation, lethargy, areflexic paralysis, seizures and coma, leukocyte dysfunction hemolysis and thrombocytopenia, as well as rickets or osteomalacia as reported in refeeding syndrome [35].

49.5.2 Neonatal Hyperphosphatemia

Hyperphospatemia is usually due to a decrease in renal function or a PTH absence (primary or secondary hypoparathyroidism) or phosphatonin deficiency. Hyperphosphatemia is most often the result of decreased renal excretion of phosphate anions as encountered in acute or chronic renal failure, particularly when glomerular filtration rate is reduced to less than 25% of normal. Hyperphosphatemia could also be the result of increased body phosphate load from blood transfusions and hyperalimentation. Increased renal tubular reabsorption of phosphate is responsible for hyperphosphatemia seen in neonatal hypoparathyroidism and associated disorders such as infants of diabetic mothers, transient neonatal hypoparathyroidism, neonatal pseudohypoparathyroidism or in babies born to mothers with hyperparathyroidism (Table 49.4). Hyperphosphatemia and secondary hyperparathyroidism have been extensively investigated as inducing factors in cardiovascular calcification. Together with passive deposition of calcium-phosphate in extraskeletal tissues, it has recently been demonstrated that inorganic phosphate induces arterial calcification directly through a real "ossification" of the tunica media [36].

49.6 Bone Mineralization

49.6.1 Factors Affecting Growth and Mineralization

During gestation, the fetus receives an ample provision of nutritional supply through the placenta. Nitrogen, energy, minerals, and vitamins allow a high velocity of body length growth, representing around 1.2 cm/week during the last trimester of gestation. The fetus maintains its hypercalcemic state in a high calcitonin and estrogen environment, promoting the modeling/remodeling ratio in favor of modeling and thus increasing endocortical bone. In addition, according to the mechanostat theory of bone development, fetal bone is also driven by the mechanical force applied to the fetal skeleton during the intrauterine resistance training provided by regular fetal kicks against the uterine wall [37, 38]. Consequently, at term the newborn skeleton has a high physical density (bone mass divided by bone volume), with elevated cortical thickness and relatively small marrow cavities.

Various factors influence the processes of growth, mineralization, and bone structure. Growth is directly related to protein and energy supplies but also to the hormonal environment comprising insulin, IGF1, and IGF2, among others. Bone formation, mineralization, and structure are related to mineral supply and hormonal factors such as PTH, PTHrP, vitamin D, and calcitonin as well as others, such as genetics and physical activity. Several factors have been found to have a significant impact on newborn bone mineral content and developing fetal bone. Reduced calcium supply, vitamin D deficiency, alcohol consumption, and smoking during pregnancy are all factors affecting fetal skeletal development in addition to low weight related to gestation and diabetes in the mother.

49.6.2 Osteopenia of Prematurity

Premature infants, particularly those born at <28 weeks' gestation, are at significant risk for reduced bone mineral content (BMC) and subsequent bone disease, variably termed metabolic bone disease (MBD), osteomalacia, osteopenia, or neonatal rickets [11, 39, 40]. Reduction in BMC and the development of MBD of prematurity are quite common among VLBW infants. However, due to the lack of widely adopted diagnostic criteria, the true incidence has not been determined. Fractures in premature infants typically occur several weeks after delivery and prior to the postnatal age of 6 months [41, 42]. Rib fractures, the most common type, usually occur silently and are diagnosed only if X-rays are performed. Therefore, the true incidence of fractures is difficult to determine, varying between 2.1% and 25% in 3 previous studies [41] conducted without the use of prospective, systematic skeletal surveys.

The risk factors commonly associated with fractures include extremely low birth weight, late (> 30 days) establishment of full enteral feeds or prolonged parenteral nutrition, exclusive use of unfortified human milk, necrotizing enterocolitis, conjugated hyperbilirubinemia, chronic lung disease, use of various medications, utilization of passive respiratory physiotherapy (i.e., chest percussion), and lack of physical activity, which may be enhanced by sedatives. Recent data on fractures among VLBW infants are still lacking, but some clinical evidence suggests that the risk for fracture is greatly reduced with the use of parenteral and enteral nutrition adapted to the special nutritional needs of the premature infant.

Risk factors for MBD are commonly encountered in the preterm infant. The majority of bone mineralization, along with calcium and phosphorus accretion, occurs during the third trimester of pregnancy. Infants born before this time thus have depleted stores of these minerals [42]. Data from bone density scans (dual-energy X-ray absorptiometry [DEXA]) performed at birth in preterm and term infants suggest that bone mineral accretion during the last trimester of gestation is higher than needed, with growth in bone volume leading to

a continuous increase in skeletal density. Preterm infants therefore have a large mineral deficit compared with term infants. Several factors increase the risk for severe MBD among VLBW infants, with the most important appearing to be an inadequate supply of calcium and phosphorus associated with the use of an enteral *vs* transplacental route. Newborn premature infants experience a diminished mineral uptake required for proper bone accretion, due, in part, to the reduced availability and to their compromised gastrointestinal (GI) absorption. Metabolic balance studies in preterm infants fed fortified human milk and formulas [1, 2] have reported that maximal calcium retention values may reach 60 to 90 mg/kg/day and maximal inorganic phosphate retention values may reach 50 to 75 mg/kg/day. The retention rates are relatively low compared with the fetal accretion rates. In total parenteral nutrition, similar data could be obtained with the use of organic phosphate supply and highly soluble calcium salt. In our unit, using calcium glycerophosphate, we provide up to 105 mg of calcium and 80 mg of inorganic phosphate/kg/day with a retention rate close to 95%.

Decreased bone mineralization and the development of osteopenia are the balanced result between two different factors, bone matrix growth directly related to energy balance and nitrogen retention on the one hand, and mineral accretion on the other hand [2, 10]. Data from DEXA scans [2, 10] performed during the first weeks after birth in both preterm and term infants suggest that bone growth, estimated by increase in bone area, is relatively higher than bone mineral accretion, leading to a continuous decrease in skeletal density (Fig. 49.6). Nevertheless, after a few weeks or months, with the continuous reduction in growth velocity, the balance is progressively reversed and the bone mass accrual compensates slowly for the early peak bone growth during the first few months of life. Physical activity appears to play a significant role in bone mineralization. During the neonatal period, mechanical strain on bone and joints stimulates bone formation and growth, whereas inactivity leads to bone resorption [37, 38]. This might be equally valid for preterm infants in incubators during the first weeks of life, who lack the *in utero* mechanical stimulation associated with regular kicks against the confining uterine wall [43]. During the initial hospitalization, the movements of preterm infants usually occur without much resistance. While in the NICU, these infants are handled with little tactile stimulation in order to reduce stressful events. Moreover, the use of drugs to reduce pain aggravates the reduction in mechanical stimulation during their stay. In order to obviate the effects of reduced mechanical stimulation, systematic physical activity programs administered several times a week by nurses, therapists, and parents have been evaluated. A number of recent studies, agree that physical activity either improves bone mineralization, as determined by single-photon absorptiometry, or increases bone formation, as estimated by the measure of serum collagen C-terminal propeptide [43]. These regimens either increased bone strength or attenuated its decrease, as evaluated by quantitative measurement of bone ultrasound transmission speed. Nevertheless, the last Cochrane review of this subject concluded that additional studies are needed before the general use of such physical activity programs can be promoted [43]. A number of other factors may also play a significant role in bone mineralization, including genetic polymorphism, mechanical stimulation, or the use of various medications that interfere with mineral absorption or retention, such as diuretics, caffeine, and corticosteroids.

Neonatal screening for MBD in preterm infants is still controversial [40]. Serum calcium levels are carefully regulated by hormonal secretion and are not a useful screening tool. However, a low serum phosphorus concentration <1.8 mmol/L) that is below the renal phosphate threshold has been related to insufficient phosphorus intake and to an increased risk for osteopenia. Urinary excretion of calcium and phosphorus has been proposed as a marker of adequate postnatal

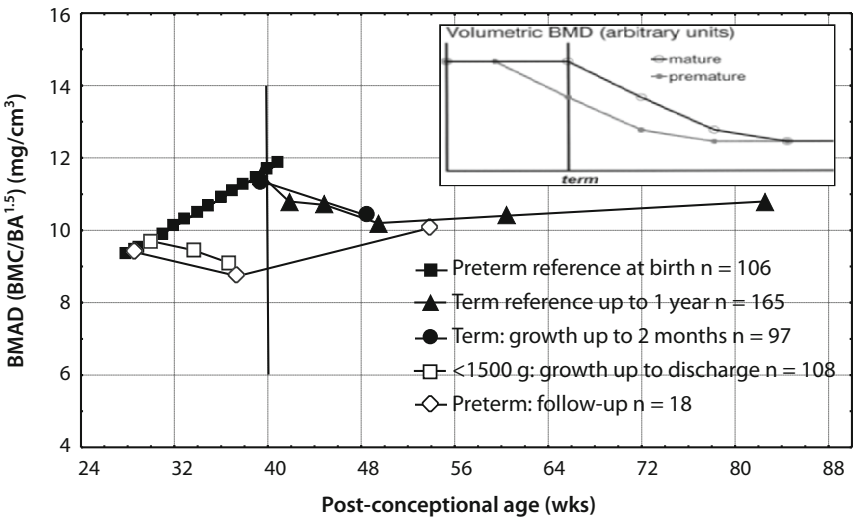

Fig. 49.6 Physiological evolution of DEXA apparent Bone Mineral Density during the last trimester of gestation and during the first year of life in healthy term infants (*dark symbols*) compared to that observed in premature infants (*open symbols*). Comparison to the evolution suggested by the mechanostat theory (*frame*)

mineralization when doses >1.2 mmol/L of calcium and >0.4 mmol/L of inorganic phosphorus are excreted simultaneously. However, these values are more appropriate for estimating the adequacy of the calcium to phosphate ratio than for estimating mineral accretion, especially when data on the mineral absorption rate are lacking.

In infants, 90% of alkaline phosphatase (ALP) is of bone origin and thought to reflect bone turnover. ALP concentrations usually increase during the first 2 to 3 weeks of life and may peak further if there are insufficient mineral supplies. Elevated levels of ALP have been reported in association with severe under mineralization based on radiologic evidence, low bone speed of sound (SOS) using quantitative ultrasound, or severe bone mineral density (BMD) deficit revealed on DEXA scans. Nevertheless, ALP is probably more sensitive for evaluating fracture risk than for assessing MBD or osteopenia.

Various radiologic investigations have been proposed for assessing bone mineralization and osteopenia among preterm infants. Plain radiography is poorly sensitive, detecting only a decrease of >20% to 40% of bone mineralization [42]. By contrast, DEXA technology, is sensitive, accurate, and precise, and its use has been validated in both preterm and term infants [1, 2, 44]. Normative data on bone mineral content, projected bone area, and BMD in healthy preterm and term infants close to birth were established in order to obtain surrogate intrauterine reference values. In addition, various indices have been proposed for reducing the anthropometric dependency of the various parameters and for facilitating group or individual comparison. Thus, data obtained from various groups allow for the determination of major changes in bone mineralization during the fetal life and postnatally in preterm and term infants. These results are in agreement with the predicted time course of volumetric bone mineral density in mature newborns and premature babies, according to mechanostat theory [39].

The use of ultrasound has been proposed for the evaluation of bone mineralization in newborn infants [40]. It is a simple, non-invasive, relatively inexpensive bedside procedure. Some machines have been designed to measure broadband ultrasound attenuation or SOS, commonly on the tibia. The propagation of sound waves in bone is determined by a number of factors, including mineral density, cortical thickness, elasticity, and micro-architecture, possibly providing a more complete picture of bone strength than measurements of BMD alone. In preterm and term infants at birth, there is a significant correlation among tibial SOS and gestational age, birth weight, birth length, and tibial length. However, the changes in SOS values during the last trimester of gestation are relatively small, accounting for only about 130 m/sec. This value is only ±1.5 times higher than the interindividual variability (standard deviation [SD] = 95 m/sec). After birth, a rapid decline in bone SOS occurs during the first days of life that cannot be completely explained by a nutritional deficit. Therefore, these data suggest that measurement of bone SOS has a lower sensitivity than DEXA for evaluating the various factors influencing bone mineralization during the neonatal period (Fig. 49.6).

In contrast to fetal bone metabolism, in which modeling is the main process inducing high net bone formation, with a rapid increase in trabecular thickness, neonatal bone metabolism is the result of a prevailing remodeling activity, defined as the cyclical succession of bone resorption and formation on the same bone surface [39, 40]. Therefore, the relative MBD of prematurity could be the result of a postnatal physiologic metabolic adaptation instead of the expression of a transitory MBD. Indeed, the relative osteopenia observed in preterm infants appears to be similar to that observed in healthy term infants during the first weeks after delivery or to that observed in early adolescence at the time of a growth spurt.

After discharge, catch-up mineralization is rapidly observed in VLBW infants [44]. At 6 months of corrected age, spine and total bone mineral density, corrected for anthropometric values, are in the range of normal term newborn infants. In fact, the catch-up mineralization observed after discharge is quite similar to that observed after the initial acceleration of growth during adolescence. Nevertheless, peak bone mass may be less during adulthood [45]. As suggested by Fewtrell and coworkers, at 8 to 12 years of age, formerly preterm infants were shorter, lighter, and had lower BMCs than controls. However, BMC was appropriate for the body size achieved and was not affected by early dieting or human milk feeding [46].

Thus, osteopenia or rickets of prematurity seems to be a self-resolving disease, although the potential long-term consequences on the attainment of peak bone mass are not clearly known. Even if BMC improves spontaneously in most infants, this discovery does not imply that a period of demineralization is acceptable. Although the long-term consequences are unclear, the benefits of prevention and treatment include avoidance of fractures and possibly improved linear growth and peak bone mass.

In summary, after birth, the development of relative osteomalacia or osteopenia is a physiologic event resulting from a mismatch of the mineral supply and the persistent growth velocity on the one hand, and from the stimulation of bone turnover as an adaptation to extrauterine life on the other. This phenomenon is enhanced in preterm infants born with low mineral stores, an immature GI tract, and reduced physical activity, who demonstrate higher growth rates than do term infants. Several of the conditions discussed above and in the articles reviewed in this chapter might increase the severity of MBD, leading to the development of severe osteopenia and the risk for fracture. Early optimal parenteral and oral nutritional support, combined with biologic neonatal screening and measurement of serum phosphorus and ALP concentrations, as well as mineral urinary excretion, appears to be helpful for the prevention of MBD. When available, DEXA is more sensitive than ultrasound for quantifying osteopenia in VLBW infants [40].

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Micronutrients and Vitamins

Olivier Claris and Guy Putet

50.1 Micronutrients

Table 50.1 summarizes the most recent recommendations [1–4].

Iron Iron (Fe) is an important micronutrient implicated in DNA replication, cellular metabolism and oxygen delivery. It is mainly involved in erythropoesis and is the first sign of deficiency in anemia, it is also implicated in neurodevelopment, and cardiac and skeletal muscle function. On the otherhand, it is a potentially toxic nutrient and, as a powerful prooxidant, it may play a major role in the oxidative stress. Furthermore, iron overload has a direct impact on cardiac and liver function. For all these reasons, the range between requirements and toxicity is narrow.

Iron is absorbed in the duodenum as ferrous. Absorption rates depend on the iron status, the form of iron given and the age of preterm (PT) infants. Iron absorption is particularly enhanced by human milk feeding and vitamin C status, and is decreased after erythrocyte transfusion and competes with zinc and copper absorption.

Iron supplementation may be started as soon as 2 weeks of age, at a dose of 2 to 3 mg/kg/day [1]. Infants receiving erythropoietin treatment require higher intake, but too high an intake may cause intestinal side effects and increase retinopathy of prematurity. It is therefore why intakes above 5 mg/kg/d are not recommended [5].

Zinc Zinc (Zn) is an ubiquitous trace metal present in numerous enzymes and participates in carbohydrate (CHO) and protein metabolism. It is required for replication, transcription and repair of DNA, and plays an important role during embryogenesis and growth. Zn is absorbed in the distal duodenum and proximal jejunum, and this is impaired by high casein intake.

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Zn deficiency is characterized by growth arrest, irritability, anorexia, alopecia, esophagitis, diarrhea and functional recommendations in immunity, skin lesions of the hands and feet and poor healing. True acrodermatitis enteropathica is rarely seen in PT infants but ELBW infants on TPN without Zn, SGA, and growing PT infants fed unfortified HM are at risk of Zn deficiency [6].

Copper (Cu) It is a component of numerous enzymes (superoxide dismutase) involved in oxidation and reduction. It helps to protect cell membranes from oxidative damage. Copper competes with Zn and iron for intestinal absorption. It is absorbed in the upper part of the intestine, and this decreases by high Zn and iron intakes. Deficiency is associated with hypochromic anemia not responding to iron supplementation, hypotonia, failure to grow, diarrhea, bone abnormalities, neutropenia. In the case of hepatic cholestasis, intake has to be reduced or stopped because of potential toxicity.

Selenium (Se) It is a glutathione peroxidase component, which protects cell membranes against peroxide induced drainage. Deficiency is only seen in infants on TPN without or with too little Se, or in Keshan disease (cardiomyopathy) areas where soil is Se deficient. Addition of Se to food or TPN must be cautious as most Se compounds are toxic if given in excess [7].

Iodine (I) It intervenes in thyroid function (T3 and T4 synthesis), and iodine deficiency (before or after birth) may impair growth and intellectual performance. Excess iodine may also create hypothyroidism. PT infants often have transitory hypothyroidism as their mechanisms to control iodine levels are immature [8]. Based on the mean content of iodine in HM some PT infants may be on negative iodine balance. Toxicity is more often due to percutaneous intake (antiseptic solution).

Chromium (Cr) It has a role in glucose homesostasis. Hyperglycemia and insulin resistance are part of Cr deficiency, never described in infants either breast or formula fed.

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 50.1 Micronutrients: Human milk (HM) content and intake recommendations [1–4]

	HM content FT	Requirements [2]		Requirements [1, 3, 4]		
		Enteral PT	Parenteral PT	Enteral PT	Parenteral FT	Parenteral PT
Iron	0.5–1 mg/L	2–4 mg/kg	0.25–0.67 mg/kg*	2–3 mg/kg	0.5–1 mg/kg	0.2 mg/kg**
Zinc	0.5–2.5 mg/L***	1–2 mg/kg	0.4 mg/kg	1.1–2 mg/kg	0.25 mg/kg	0.45–0.5 mg/kg
Copper	600–800 µg/L	120–150 µg/kg	20 µg/kg	100–132 µg/kg	20 µg/kg	
Selenium	15–20 µg/L	1.3–4.5 µg/kg	1.5–4.5 µg/kg	5–10 µg/kg	2–3 µg/kg	
Iodine	70–90 µg/L	10–80 µg/kg	1 µg/kg	11–55 µg/kg	1 µg/day	
Chromium	0.3–0.5 µg/L	0.1–2.25 µg/kg	0.05–0.3 µg/kg	0.03–1.23 µg/kg	not necessary	
Manganese	5 µg/L	0.75–75 µg/kg	1 µg/kg	<27 µg/kg	1 µg/kg	<50 µg/day
Molybdenum	2 µg/L	0.3–4 µg/kg	0.25–1 µg/kg	0.3–5 µg/kg	0.25 µg/kg <5 µg/day	1 µg/kg
Fluorine				1.5–60 µg/kg		

* After 2 weeks of life. ** Unnecessary before 3 weeks. *** Decrease with postnatal days.

Manganese (Mn) It is a component of several enzymes acting in gluconeogenesis, mitochondrial membrane maintenance and in mucopolysaccharide synthesis. Mn deficiency has not been described in human conclusively, however Mn toxicity has been seen in adults with extrapyramidal symptoms. Advisable intakes are based on what is contained in human milk.

Molybdenum (Mo) It intervenes in the function of xanthine, aldehyde and sulfite involved in purine metabolism and sulfur excretion. Deficiency is only described in adults on very long term TPN. Advisable intakes are based on what is contained in HM.

Fluorine (F) It is found in bones and teeth mainly. There are no data to make any recommendation except that it is found in human milk and crosses the placenta.

50.2 Vitamins

Table 50.2 summarizes the most recent recommendations for vitamin intake.

Vitamin B1 (**thiamine**) Vit B1 is a coenzyme essential for CHO metabolism and lipids synthesis once transformed by the liver into thiamine pyro phosphate. It is absorbed in the proximal small intestine. Deficiency is known as "beriberi". A thiamine deficient total parenteral nutrition (TPN) may induce severe lactic acidosis and death.

Vitamin B2 (**riboflavine**) Vit B2 is implicated in energy metabolism (it forms flavin, adenine, dinucleotides). It is absorbed in the small intestine. Deficiency leads to stomatitis, dermatitis and anemia. Requirements are based on HM content and are related to protein intake. If protein supply is given without Vit supplementation, a deficit can occur [9].

Vitamin B3 (**niacin**) Vit B3 can be synthesized from tryptophane (Try) in the presence of B6, once this amino acid (AA) exceeds minimal intake (60 mg of Try → 1 mg of niacin expressed as Niacin Equivalent). It is also a cofactor for electron transport and energy metabolism. Absorption is effective in the small intestine. Deficiency is known as pellagra (dermatitis, diarrhea and neurological symptoms). It usually results from multiple deficiency and poor protein intake not observed in newborn infants.

Vitamin B6 (**pyridoxine**) Vit B6 has a role in the metabolism of AA, prostaglandins, CHO, in the development of immune system and neurologic function. Its requirement is related to protein intake (15 µg of B6 should be available per g of protein). It is absorbed in the small intestine. Deficiency leads to vomiting, irritability, dermatitis, failure to thrive, hypochromic anemia and neurological symptoms as convulsions.

Vitamin B9 (**folate**) Vit B9 is active in the biosynthesis of purines, pyrimidine and AA metabolism. Its activity decreases by Zn deficiency. Deficiency is associated with megaloblastic anemia, leucopenia, thrombocytopenia, growth insufficiency, and lesions in small intestine, mostly in context of malabsorption syndromes.

Vitamin B12 (**cobalamin**) Vit B12 is involved in DNA nucleotides synthesis. Absorption depends on gastric pH and occurs in the small intestine. Deficiency of cobalamin is known as leading to megaloblastic anemia, glossitis and neurologic signs, but although this is not seen in FT breast fed infants, it is well established in infants of vegetarian mothers.

Vitamin B5 (**pantothenic acid**) It is a precursor of Coenzyme A (energy metabolism). It is absorbed in the small intestine. Deficiency has not yet been reported as diet provides sufficient amounts.

Table 50.2 Vitamins: human milk (HM) content and intake recommendations [1, 2]

	HM content FT	Requirements [2]		Requirements [1]	Equivalents
		Enteral PT	Parenteral PT	Enteral PT	
Vit A	660–1000 IU/L	750–1500 IU/kg	750–1500 IU/kg	400–1000 µg RE/kg	1 RE = 1 µg all trans retinol 1 RE = 3.33 IU Vit A 1 RE = 6 µg β carotene 1 RE = 12 µg other carotenoids
Vit D	20–30 IU/L	200–1000 IU/day	60–400 IU/day	800–1000 IU/day	1 µg cholecalciferol = 40 IU Vit D
Vit E	3–4 IU/L	6–12 IU/kg	2.8–3.5 IU/kg	2.2–11 mg/kg TE	1 IU = 1 TE 1 IU = 0.67 mg α-tocopherol 1 IU = 1 mg dl-α-TA
Vit K	5–10 µg/L	8–10 µg/kg	10 µg/kg	4.4–28 µg/kg	

IU international units, *RE* retinol equivalent, *TE* tocopherol equivalent, *TA* tocopherol acetate.

Table 50.3 Vitamins: effect of temperature and light, and toxicity

	Temperature	Light	Toxicity
Vit A	↓	Photodegraded	Intra cranial hypertension if >5000 IU/day
Vit D	Stable	Stable	Hypercalcemia, hypercalciuria, anorexia, vomiting, failure to thrive, calcifications
Vit E	Stable	Slightly affected	Large doses associated with sepsis, NEC
Vit K	Stable	↓	Not reported

Table 50.4 Vitamins: daily intake recommendations [1, 2]

	Requirements [2]				Requirements [1]	
	Enteral FT	Enteral PT	Parenteral FT	Parenteral PT	Enteral PT	Parenteral FT
B1	30 µg/kg	300 µg/kg	1–2 mg/day	350 µg/kg	140–300 µg/kg	350–500 µg/kg
B2	40 µg/kg	450 µg/kg	150 µg/kg	150 µg/kg	200–400 µg /kg	150–200 µg /kg
B3	0.2 mg/kg	4.5–6 mg/kg	17 mg/day	5 mg/kg	0.3–5 mg/kg	4–6.8 mg/kg
B6	14 µg/kg	180–300 µg/kg	1000 µg/day	180 µg/kg	45–300 µg/kg	150–200 µg/kg
B9	9.4 µg/kg	45–50 µg/kg	140 µg/day	56 µg/kg	35–100 µg/kg	
B12	0.05 µg/kg	0.3 µg/kg	0.75 µg/day	0.3 µg/kg	0.1–0.77 µg/kg	0.3 µg/kg
B5	1.7 mg/day	2 mg/day	5 mg/day	2 mg/kg	0.33–2.1 mg/kg	1–2 mg/kg
B8	0.7 µg/kg	4–40 µg/kg	20 µg/day	6 µg/kg	1.7–1.65 µg /kg	5–8 µg /kg
Vit C	6 mg/kg	30–40 mg/kg	80 mg/day	25 mg/kg	11–46 mg/kg	15–25 mg/kg

Table 50.5 Vitamins: human milk (HM) content, effects of temperature and light, and toxicity

	HM	Temperature	Light	Toxicity
B1	165–220 µg/L	↓	Photo degraded	Only in adults
B2	350–575 µg/L		↓	Not clearly defined
B3	1.8–2.5 µg/L			No side effect with nicotinamide
B6	130–310 µg/L		Inactivated	Very rare only in adults
B9	80–135 µg/L	Destroyed	Inactivated	Very rare may mask Vit B12 deficit. May depress Zn absorption
B12	0.2–1 µg/L			Not reported
B5	2–2.5 mg/L			Not reported
B8	5–9 µg/L			Not reported
Vit C	35–85 mg/L	Inactivated		Rebound scurvy in newborn only after large doses during pregnancy

Vitamin B8 (biotin) Except in some metabolic diseases, deficiency is not seen in enterally fed infants as it is synthesized in gut, but is seen in TPN (pallor, anemia, dermatitis, lethargy, EEG abnormalities). It is also absorbed in the small intestine.

Vitamin C (ascorbic acid) Vit C has a role as a cofactor in hydroxylation reactions (proline, lysine, norepinephrin synthesis and Try) and as an antioxidant. It also has a role in folic acid conversion to folinic acid (active form) and in the oxidation of tyrosine (Tyr), and in iron absorption. 1 IU of L Ascorbic Acid corresponds to 50 μg. It is absorbed in the small intestine. Deficiency is known as scurvy. A transient elevation of plasma Tyr and Phenylalanine has been reported in VLBW infants fed high protein casein formulae.

Vitamin A This term refers to compounds (retinoids) having similar activities and structure as retinol (natural molecule derived from beta-carotene). Vit A activity is expressed as RE (retinol equivalent), 1 RE = 1μg retinol = 3.3 IU Vit A. Vit A is mainly stored in liver and circulates in blood linked to retinol binding protein (RBP), and is implicated in protein synthesis and epithelial cell functions, growth and immune functions. It has also strong antioxidant properties. It is absorbed in the upper part of the small intestine. No clinical deficiency is described in FT breastfed infants and precise requirements for PT infants are still unknown with recommended intakes are based mainly on biological data. Vit A supplementation may play a role as an antioxidant factor, and as a protective factor for bronchopulmonary dysplasia (BPD) [10].

Vitamin E The term of Vit E refers to 8 compounds having similar activities. The most active compound is α-tocopherol, which shows a strong antioxidant capability by protecting from lipid peroxidation. Vit E is entirely expressed as α-tocopherol equivalent (αTE), 1 mg αTE = 1 mg d-α-tocopherol = 1.49 IU, and 1 IU = 1 mg dl-α-tocopherol acetate. Vit E requirements depend on polyunsaturated fatty acid intake. It is absorbed in the small intestine. Hemolytic anemia is the main consequence of Vit E deficiency. Some controversy still persists upon the role of Vit E in BPD and retinopathy of prematurity. Large intakes of Vit E (above 50 mg/kg) have been implicated in gastro intestinal adverse effects and sepsis [11].

Vitamin K There are to 2 forms: Vit K1 (phyloquinone: plant form) and Vit K2 (menaquinone: synthesized by bacteria). Vit K is necessary for hepatic synthesis of coagulation factors (II, VII, IX, X, protein C and protein S). It is absorbed in the small intestine. It is needed in a very small amount and, as it is synthesized partly in the gut, synthesis is less pronounced with HM, and deficiency is seldomly seen, except in neonates and in cases of malabsorption or hepatic disease [10].

Vitamin D Along with calcium and phosphorus homeostasis (see also Chapter 49), Vit D plays a physiologic role in neuromuscular function and cell growth and differentiation. It is absorbed in the small intestine. As Vit D deficiency in pregnant women is still frequent, recommended supply for neonates needs to be given as soon as enteral feeding is established [12].

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Safety of Medications During Pregnancy and Breastfeeding

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51.1 Introduction

The use of medications during pregnancy has become a concern since the thalidomide tragedy. However, although avoiding medications during pregnancy and breastfeeding may be desirable, it is often not possible. Medication use during pregnancy and postpartum is often necessary and unavoidable in chronic conditions such as asthma, diabetes mellitus, hypertension, epilepsy or depression. Women can also develop acute illnesses or pregnancy-induced complications that necessitate drug therapy. In addition, because approximately 50% of pregnancies are unplanned, women are frequently exposed to therapeutic drugs not necessarily intended to be used during pregnancy. Epidemiological studies have determined that two-thirds of all pregnant women use at least one prescription drug during pregnancy [1, 2]. However, in a review conducted in 2001 it was estimated that more than 90% of the drugs approved by the FDA between 1980 and 2000 had insufficient human pregnancy data to determine whether the benefits of treatment exceeded the risk to the embryo and/or fetus [3]. This reality highlights the growing need for more and better data regarding medication use during pregnancy and breastfeeding.

To guide physicians in the interpretation of the teratogenic risk associated with prescription drugs, in 1979 the FDA created a rating system that classifies drugs on the basis of data from animals and humans, ranging from class A drugs, which are designated as safe for use during pregnancy, to class X, which are contraindicated during pregnancy because of proven teratogenicity. However, this system oversimplifies the issues relevant to prescribing a medication to a pregnant patient, and has been criticized as inconsistent, misleading and of limited value. The FDA has recognized the limitations of the current system, and a new approach to pregnancy and lactation labeling is under development. The proposed change to the FDA labeling system will provide more detailed, comprehensible, and practical information about the use and effects of drugs during pregnancy and lactation [4].

This chapter reviews the issues related to safety of medications use in pregnancy and breastfeeding describing the principles of teratogenicity assessment and providing practitioners with resources for obtaining up-to-date information regarding exposures during pregnancy and lactation.

51.2 Establishing the Risk – The Importance of Observational Data

When a drug is first marketed, there are usually no human data on drug exposure in pregnancy. Due to ethical concerns about possible harm, pregnant and breastfeeding women are traditionally excluded from clinical trials. Although animal studies function as a screen for potential human teratogenicity and are a required part of the drug development process, they do not necessarily predict the effects in humans because of considerable variations in species-specific effects [5]. In the absence of randomized controlled trials, the best and only ethical way to achieve better knowledge of possible teratogenic effect of drugs during pregnancy is collection and follow-up of observational data [6].

51.2.1 Case Reports and Epidemiological Studies

How do we establish that specific agents are teratogens? Evidence of teratogenicity in humans frequently starts from a single case-report or case series. Case reports can be very useful, or useless, depending on the relatively simple statistical consideration: If a specific, rare malformation occurs in

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

association with a drug that is taken rarely in pregnancy, then a few case reports may prove causation [7]. For example, the first few cases of anotia (lack of ears) and brain defects after isotretinoin have established causation, because the number of cases by far exceeded the rate of these rare malformations [8]. If, on the other hand, the drug is taken by many pregnant women or the malformation in question is common (e.g., ventricular septal defect), then case reports are useless, as they may simply reflect the spontaneous occurrence of malformations in the general population. Case reports have high sensitivity but low predictive value and are thus limited in proof of causation. Hence one must be careful to not rely solely on this type of published information.

At this point, epidemiological cohort and case control surveillance studies are the main methods for verifying an existing risk. Cohort studies are typically designed to determine whether women who took a specific drug during pregnancy have a larger number of malformed children than women who did not use the same drug. Cohort studies are usually prospective (i.e., subjects are enrolled before the outcome is known) and are commonly used for evaluating risk, but their size is frequently small and the selection of appropriate comparison groups (unexposed pregnancies) is important. Case-control studies are based on the ability to link specific malformations with gestational exposure to the drug in question. They are useful when there has been extensive use of a drug by pregnant women or when a drug is suspected of causing relatively rare malformations.

Pregnancy exposure registries are a type of cohort study that prospectively enroll pregnant women exposed to a specific drug of interest and evaluate the associated pregnancy outcomes. They are increasingly used for evaluating risk of newly marketed drugs, but sometimes are limited by the absence of important information such as the timing and dose of the exposure, and the lack of an appropriate comparison group [9].

Databases developed for administrative purposes are also frequently used for both identifying women who were exposed to a specific drug during pregnancy and the outcomes of their pregnancies. These data can be used to identify high-risk teratogens, although for recently introduced drugs they are likely to provide results more slowly than pregnancy registries [10].

Table 51.1 Drugs with potential teratogenic effects [25–27]

Drug	Teratogenic effect
Aminopterin	CNS, limb and skeletal malformations
Angiotensin-converting-enzyme inhibitors	Oligohydramnios, IUGR, neonatal renal failure, anuria, decreased skull ossification, pulmonary hypoplasia, joint contractures, death
Carbamazepine	Neural tube defects, facial dysmorphism
Cyclophosphamide	Skeletal, palate and ocular defects, IUGR
Danazol	Virilization of female fetus
Diethylstilbestrol	Vaginal/cervical carcinoma and other genitourinary defects in female and male offspring
Fluconazole (high doses 400-800 mg/day)	Brachycephaly, abnormal facies, abnormal calvarial development, cleft palate, arthrogryposis, CHD
Lithium	Cardiac defects, particularly Ebstein's anomaly of the tricuspid valve
Methimazole	Aplasia cutis, choanal atresia, esophageal atresia, hypoplastic nipples, scalp defects
Methotrexate (≥12.5 mg/wk)	Large fontanelles, abnormal head shape, craniosynostosis, ocular and skeletal defects
Misoprostol	Moebius syndrome, terminal transverse limb defects, arthrogryposis
Mycophenolate mofetil	Microtia, cleft lip/palate, hypoplastic fingers and toenails, heart defects, micrognathia
Penicillamine	Cutis laxa/connective tissue anomaly
Phenytoin	Fetal hydantoin syndrome: ocular hypertelorism, flat nasal bridge, hypoplastic nails/distal phalanges, microcephaly, developmental delay
Systemic retinoids (isotretinoin, etretinate)	CNS, microtia/anotia, craniofacial, cardiovascular, eye anomalies, limb defects
Tetracycline	Dental staining
Thalidomide	Limb-shortening defects, internal organ defects
Trimethadione/ Paramethadione	IUGR, facial and CNS defects
Trimethoprim	Neural tube defects
Valproic acid	Neural tube defects, fetal valproate syndrome (distinct craniofacial appearance, limb defects, heart defects, a cluster of minor and major anomalies, and developmental delay)
Warfarin	Skeletal and CNS defects, Dandy–Walker syndrome

CNS Central nervous system, *IUGR* Intrauterine growth restriction, *CHD* Congenital heart disease.

51.3 Human Teratogenesis

Teratogenesis is defined as structural or functional dysgenesis of the fetal organs [11]. Adverse pregnancy outcomes as a result of teratogen exposure include structural malformations, embryonic or fetal death, growth alteration, and functional/neurobehavioral deficits [11].

The incidence of major congenital malformations in the general population is 1–3% of all births. For the majority of birth defects (65–75%) the etiology is unknown [12]. In 15-25% of cases the cause is attributable to genetic or chromosomal abnormalities, while 10% of birth defects have an exogenous and potentially preventable cause, such as maternal diabetes, alcohol abuse and therapeutic drugs. The part played by drugs is low, and probably account for less than 1% of all birth defects [12].

One of the common misconceptions about a teratogen is that exposure to that agent during pregnancy will always result in abnormal fetal development. However, even high-risk teratogens, such as thalidomide and isotretinoin, do not inevitably cause birth defects and they typically affect one out of every four exposed fetuses. With the exception of thalidomide and isotretionoin, the other human teratogens are associated with ≥90% normal infants in exposed pregnancies. Fifty years after thalidomide-associated embryopathy, only about 20 drugs or groups of drugs have been established as human teratogens when used in clinically effective doses (Table 51.1).

51.4 Basic Principles of Teratology

An understanding of the principles of teratology is critical to determine if an agent may be the cause of an adverse outcome. These principles help provide the biologic plausibility component needed when one is assessing teratogenicity. Factors that may influence whether a teratogen will actually produce teratogenic outcomes in a particular case include the gestational timing of exposure, dose, route and duration of exposure, the nature of the agent itself, concurrent exposures to other agents, underlying maternal diseases, and genetic susceptibility of the mother and the embryo or fetus [13].

51.4.1 Timing of Exposure

Among the criteria of human teratogenicity, one of the mot important is that of time; that is, the evidence that exposure to teratogenic agents occurred during organogenesis of the given organ or part of the body. The most vulnerable period of organogenesis is from the third through to the ninth week after conception (embryonic stage). Each of the developing organ systems undergoes one or more critical processes during this stage and interference in these processes has the greatest likelihood of causing a structural anomaly. The vast majority of malformations seen in the newborn have already occurred by the end of 8 weeks.

A drug exposure that occurred after the critical period could not produce a birth defect. For instance, the neural tube defect results from events that occur before day 28 following conception. After day 28, the neural tube is closed, and malformations of closure can no longer occur. Thus, during the evaluation of a pregnancy affected by a NTD, it is essential to determine if potential exposures occurred before this time critical period. Examples of a few other anomalies and the post-conception days before which the causative event or exposure must have occurred are cleft lip (36 days), cleft palate (8 weeks), ventricular septal defects (6 weeks), and hypospadias (12 weeks) [11].

The fetal phase, from the end of the embryonic stage to term, is the period when growth and functional maturation of organs and systems already formed occurs. Teratogenic exposure in this period may affect fetal growth and the size or function of specific organs, rather than gross structural anomalies.

On the other hand, exposure to a teratogenic agent before implantation, which occurs 8–10 days after conception in most cases [14], is unlikely to result in malformation. This is known as the "all-or-none" period, as insults to the embryo are likely to result in either death of the conceptus and miscarriage (or resorption), or in intact survival. It is biologically plausible that exposure to teratogens during the preimplantation stage does not cause congenital malformations, unless the agent persists in the body beyond this period.

51.4.2 Exposure Dose

A broadly accepted principle of teratology is that there is a dose-effect relationship with developmental toxicity. There seems to be threshold levels for most human teratogens at which no adverse outcomes are known to occur if exposure is limited to subthreshold levels. An agent may be safe at one dosage, but induce birth defects at higher dosages. However, there are only few examples of drugs in which the dose has been proven to be a major determinant of their teratogenicity in humans. As an example we could use fluconazole, a triazole antifungal agent that has been used in the treatment of vaginal candidiasis and other fungal infections. A single fluconazole dose of 150 mg, usually prescribed for vaginal candidiasis, has not been associated with increased risk for malformations at any point during gestation [15]. On the other hand, five case reports describing multiple congenital anomalies (a distinct and consistent pattern of malformations) in infants born to mothers treated with high dose fluconazole (400–800 mg/day) during first trimester of pregnancy have been published [16, 17].

Although a precise dose-response curve cannot be determined, fluconazole is only teratogenic at these high, continuous doses.

Nevertheless, the basic principle of dose related teratogenicity of therapeutic drugs seems to be more complex, as illustrated by the axiom that "a teratogenic response depends upon the administration of a specific treatment of a particular dose to a genetically susceptible species when the embryos are in a susceptible stage of development" [11].

The teratogenicity of an exposure is influenced by both the maternal and fetal genotypes, which can result in differences in cell sensitivity, placental transfer, metabolism, receptor binding, or drug distribution. The mother metabolizes some medications extensively; their teratogenicity depends upon whether a toxic form reaches the embryo or fetus in sufficient quantities to produce adverse effects.

51.5 Teratogen Risk Counseling

It is important for pregnant women to know that even without any drug exposure there is 1–3% baseline risk of major congenital malformations among all pregnancies. Without appropriate evidence-based counseling, women may perceive the risk of taking a medication during pregnancy or lactation to be greater than the actual risk [18]. As a result, they may undermine their own health or that of their child by discontinuing needed medications, discontinuing breastfeeding despite its known health benefits, or terminating a pregnancy [19].

The selection of drugs for pregnant women or women planning pregnancy needs to be supported by the available relevant information about their safety in pregnant women [20]. Adequate knowledge on potential teratogenicity of a drug permits modification of therapy before conception. If the dilemma is between 2 or more drugs of the same group, the one with better evidence of safety should be selected. In general, the rule of thumb during pregnancy remains; choose an older agent for which there are more fetal safety data. A proactive teratogen risk counseling should include a critical appraisal of the published information and an evaluation of the consequences in the mother and her fetus if the treatment continues or if it stops. Evidence-based counseling ensuring that the potential risks of maternal drug therapy is balanced against the maternal-fetal risks of the untreated condition are the mainstay of teratogenic risk counseling [20].

Most physician and other health professionals receive insufficient training to answer all the questions pregnant women ask about the effects of chemical, physical, or infectious agents on the developing embryo or fetus. To receive up-to-date, evidence-based information on the safety and risks of drugs during pregnancy and lactation, physicians can consult a teratogen information services or on-line databases listed in Table 51.2.

51.6 Breastfeeding and Medications

Breast milk possesses nutritional and immunologic properties superior to those found in infant formula. World Health Organization actively promotes breastfeeding as the best source of nourishment for neonates and young infants and advises exclusive breastfeeding for the first 6 months of age, with continued breastfeeding along with appropriate complementary foods up to two years of age or beyond [21]. Breastfeeding has become more common in recent years, due in part to the many known health benefits to babies and mothers.

It is generally accepted that all medications transfer into human milk to some degree, although it is almost always quite low [22]. Although the majority of medications are considered to be compatible with breastfeeding [23], there have been many case reports of clinically significant toxicity in breastfed infants from some medications used by the mother [24]. Unfortunately there is a paucity of epidemiologic data regarding the risk of adverse effects in infants as a result of exposure to drugs in breast milk.

The transfer of medications across the basal membrane of the mammary gland alveoli depends on lipophilicity, protein binding, molecular weight and on the degree of substance ionization. The dose of a drug an infant receives during breastfeeding depends on the amount excreted into the breast milk, the daily volume of milk ingested, and the average plasma concentration of the mother. An important factor to consider is also the infant clearance, which is itself dependent on the ontogeny of elimination pathways and pharmacogenetics. One of the more popular methods for estimating exposure of the breastfed neonate to a specific drug is to determine the Relative Infant Dose (RID) which represents the weight corrected percent of the maternal dose ingested by an exclusively breastfed neonate. The RID gives the clinician a feeling for just how much medication the infant is exposed to on a weight-normalized basis. It is generally accepted that for drugs without excessive toxicity a relative infant dose of less than 10% is considered safe for full-term infants [22].

A practical view to take when prescribing drugs during breastfeeding is to choose drugs with short half-lives, high protein binding, low oral bioavailability, high molecular weight, and RID less than 10%, as well as drugs for which there are published data, rather than those introduced recently [22]. Greater precaution is advised when prescribing

Table 51.2 Resources for information on medication use in pregnancy and breastfeeding

Books	Briggs GG (2011) Drugs in Pregnancy and Lactation, 9th ed. Lippincott, Williams & Wilkins, Philadelphia Hale TW (2010) Medications and Mothers' Milk, 14th ed. Hale Publishing, Amarillo, Texas
On-line databases	Reprotox: http://reprotox.org TERIS: http://depts.washington.edu/terisweb/teris/ LactMed: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?LACT
Teratology Information Services	OTIS: http://www.otispregnancy.org/hm/ Motherisk: http://www.motherisk.org ENTIS: http://www.entis-org.com/index.html
Pregnancy Registries	www.fda.gov/womens/registries

medications for mothers of premature or otherwise compromised infants or newborns in the first weeks of life than for older, healthy infants. It is tremendously important to always evaluate the infant's ability to handle small amounts of medications. Because many drugs require renal and/or hepatic excretion, clearance may be slowed in preterm infants, who have immature renal and hepatic function.

The American Academy of Pediatrics publishes periodic statements on the transfer of drugs into breast milk [23]. Drugs are listed based on their safety for nursing infants. Most medications that are listed are included in the safest category - Maternal Medication Usually Compatible with Breastfeeding. Limitations of these recommendations include infrequent updates (last update is from 2001), little detail on the medications and omission of many medications. Table 51.2 lists resources that contain more comprehensive information. The on-line database LactMed can be especially useful in the office setting because it is regularly updated and easily accessible without subscription.

51.7 Resources and Accessibility of Information

Multiple resources for obtaining up-to date, evidence-based information regarding exposures during pregnancy and lactation are available, including books, on-line databases and teratology information services (Table 51.2).

Among books, *Drugs in Pregnancy and Lactation* by Briggs et al [25] is a highly used reference guide. A fetal and breastfeeding summary and recommendations are given for about 1200 medications. Another useful book is *Medications and Mothers' Milk*, a comprehensive reference on the impact of more than 1000 currently used medications on breastfeeding mothers and infants, written by Hale [22].

The online REPRORISK system (available from Micromedex) provides access to teratogen information databases REPROTOX and TERIS. Online access to these databases is available for a subscription fee. REPROTOX is an updated, comprehensive database providing summary information to health care professionals on the effects of chemical and physical agents on fertility, pregnancy and lactation [26]. There are summaries for more than 4000 agents, along with references for the data included.

TERIS is another online database designed to assist physicians or other healthcare professionals in assessing the risks of possible teratogenic exposures in pregnant women [27]. The database consists of a series of agent summaries, each of which is based on a thorough review of published clinical and experimental literature.

LactMed (Drugs and Lactation Database) is a peer-reviewed and fully referenced database of drugs to which breastfeeding mothers may be exposed. Among the data included are maternal and infant levels of drugs, possible effects on breastfed infants and on lactation, and alternate drugs to consider. It is available without subscription at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?LACT

In addition to the cited resources, healthcare providers also may obtain information from, or refer pregnant and breastfeeding patients to, a teratology information service for information and counseling about medication exposures.

OTIS (Organization of Teratology Information Specialists), located in the United States and Canada, provides accurate, free, evidence-based, clinical information to patients and health care professionals about exposures during pregnancy and lactation. On the OTIS website (http://www.otispregnancy.org/hm/) is information about the organization, downloadable fact sheets on more than 70 common pregnancy exposures available in English, Spanish, and French, and access to current teratology research studies.

ENTIS is European Network of Teratology Information Services, similar to OTIS (http://www.entis-org.com/index.html).

A list of many of the existing postmarketing pregnancy registries is maintained by the Office of Women's Health, US Food and Drug Administration at www.fda.gov/womens/registries

51.8 Conclusions

When considering the safety of drugs in pregnancy and breastfeeding it is necessary to make a risk-benefit calculation with often insufficient data about the risk. The term "safety" implies the absence of risk, which is impossible to demonstrate conclusively with any kind of epidemiological studies. Nevertheless, only a limited number of drugs have been proven to cause malformations in humans, the vast majority of drugs taken by pregnant women do not pose a significant risk when used in recommended doses. Although decisions about medication use must take into account the potential risk to the embryo, fetus, and infant, consideration of the impact on the mother's health is equally important. Many maternal diseases can be markedly improved by appropriate therapeutic interventions. Withholding of treatment because of theoretical concerns for the fetus is justifiable for limited number of drugs, and even then it should be the mother's decision.

It is crucial that physicians provide women with balanced, evidence-based information concerning drug exposure during pregnancy and breastfeeding as misinformation and unrealistic perception of the teratogenic risk of medications may lead to inadequate treatment of maternal disease, discontinuation of breastfeeding or unnecessary termination of pregnancies.

For obtaining up-to date, evidence based and easy to access information we refer you to multiple resources listed in Table 51.2.

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Developmental Pharmacology and Therapeutics

Erika Crane, Victoria Tutag Lehr, Merene Mathew and Jacob V. Aranda

52.1 Introduction

Developmental pharmacology and therapeutics is a broad field that includes the study of drugs during growth and development and the effect of these drugs on development itself. Development is a continuum that includes periods of conception, fetal stages and the neonatal and childhood phases of development. This chapter focuses on pharmacology and therapeutics in the newborn infant.

In practice, newborn infants are exposed to many drugs via transplacental transfer to the fetus, as well as neonatal exposure in newborn nurseries and intensive care units. Drug utilization surveys indicate at least 400 drugs are used in newborns, with pervasive polypharmacy use in sick newborns [1, 2]. Early pharmacologic surveys suggested that a neonate receives an average of about 7 drugs [2–4]. However, recent pharmacoepidemiologic surveys suggest that a sick newborn in the intensive care unit receives about 7 to 71 different drugs during their hospital course. At least 80% of these drugs have not been adequately studied in newborns and are used "off-label" in this population since the basic clinical pharmacology data including pharmacokinetics and drug elimination are not available [4–6]. Heterogeneity mainly due to fetal and post-neonatal maturity in the neonatal population complicates safe medication use. A term infant differs from a very low birth weight newborn in their ability to metabolize and eliminate drugs. Moreover, advancing age after birth influences drug clearance from the body and drug dosing regimens. Fig. 52.1 illustrates the changes in plasma half lives of a drug (theophylline) as a function of age. Another example is shown in infants less than 2 years of age with much slower valproate elimination (a commonly prescribed antiepileptic drug for pediatric patients) compared with adults and older children [7]. Children aged 2 to 10 years have plasma clearances of valproate, which are 50% higher than adults. After 10 years of age, pharmacokinetic parameters approach that of adult patients for this drug. Careful monitoring and titration of drug dosage, serum concentration, signs of toxicity and clinical effect are important as a child develops. Generalization that drug metabolism and elimination is markedly slower in the newborn infant relative to older children and adults is not always applicable. For instance, plasma clearance of a new echinocandin, micafungin, is much faster in preterm newborns than older children and adults [8] and hence will require substantially higher daily doses of micafungin [9, 10]. Drug dosages for many drugs used in neonates are indicated in other chapters of this book. However, changes in drug disposition, drug action and dosing requirements during the neonatal period are discussed in this chapter.

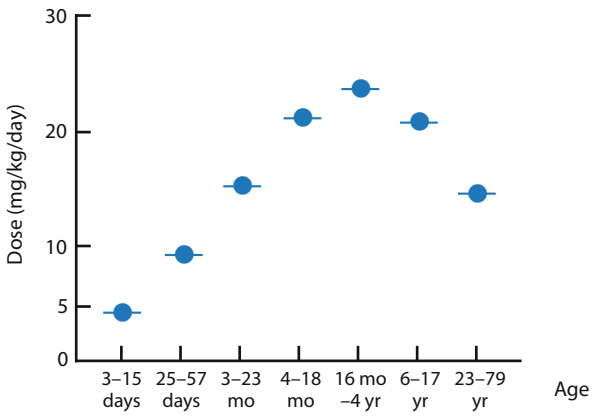

Fig. 52.1 Theophylline dose to mantain plasma concentration ≅ 10 mg/L

52.2 Developmental Pharmacokinetics

As neonates and children grow and mature, physiologic and biochemical developmental changes alter how the body handles and responds to a drug. These dynamic changes often do not have a linear relationship with such factors as age or

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

weight. Within the neonatal population, great variability in gestational age, development and weight results in highly varied dosing regimens of the same drug (Table 52.1) [11]. Developmental pharmacokinetics refers to these physiologic parameters, which define how an infant or child absorbs, distributes, metabolizes and eliminates drugs.

52.2.1 Absorption

Changes in absorptive surfaces can modify the rate and bioavailability of a drug [12]. At birth, the gastric pH is neutral and remains relatively high throughout infancy, which may effect the ionization and stability of the drug. Acidity is further decreased by the frequent ingestion of alkaline milk products and decreased gastric acid production [13]. Gastric acid production does not reach adult levels until 3 years of age [14]. Weak acids such as phenobarbital will have decreased absorption, while weak bases such as diazepam have increased absorption. Acid labile drugs such as penicillins are less likely to be degraded, therefore have increased bioavailability [14].

Most absorption of orally administered drugs occurs in the duodenum. Gastric emptying and intestinal transit time determine the rate at which a drug reaches the small intestine and for how long the drug stays in contact with the absorptive surfaces [12]. In general, gastric emptying is usually delayed in infants, not reaching adult rates until 6–8 months of age, which may delay drug absorption [14]. Intestinal motility is often erratic in childhood, with neonates and infants having a prolonged transit time and toddlers having relatively rapid intestinal motility compared with older children and adults [14]. Intestinal flora, ontogeny of intestinal metabolic enzymes and developing molecular transport mechanisms may also contribute to the erratic and often slower absorption of drugs in the neonatal gastrointestinal tract [12].

Alterations in neonatal drug absorption may occur with other routes of administration. Absorption is often enhanced for drugs administered topically, secondary to thinner stratum corneum and increased total body surface area to body mass ratio in the neonate [12]. Developmental changes affect the skin throughout infancy and childhood, influencing the rate and extent of absorption, metabolism, and bioavailability of topical medications. Skin accounts for up to 13% of an infant's total body weight compared with only 3% of an average adult's body weight [15]. This greater total body surface area ratio to body mass results in a much greater proportion of drug absorbed per kilogram of body weight for infants compared with adults [16]. Therefore, infants and young children may develop toxic drug serum concentrations with topical administration of medications [16]. Hydration of the stratum corneum is greatest in the axillae, diaper area, antecubital space and popliteal fossae, enhancing permeability. Absorption of intramuscularly administered drugs also varies in infancy, and is influenced by skeletal muscle blood flow and density of skeletal muscle capillaries [12]. The intramuscular route of drug administration is also painful and is best avoided.

Table 52.1 Neonatal dosing of gentamicin based on weight and age

Age	Weight (g)	Dose (mg/kg/day)	Interval (h)
Premature neonates	<1000	3.5	24
	<1200	2.5	18–24
Term neonates			
<7 days of age	1200–2000	2.5	12
>7 days of age	1200–2000	2.5	8–12
	>2000	2.5	8

Data from [11].

52.2.2 Distribution

Distribution of drugs within the body is greatly influenced by developmental changes in body composition. Neonates have a higher total body water content of 80%, which quickly declines by 4 months of age to the adult fraction of 60%. Neonates also have a higher fraction of extracellular water, which steadily declines throughout childhood. Hydrophilic drugs such as gentamicin or amikacin often have a higher volume of distribution in neonates, resulting in larger doses to obtain a therapeutic serum concentration. Fatty connective tissue is the major component of the subcutaneous layer and begins to accumulate around week 14 of gestation [17, 18]. The fat content in the neonate is relatively low, increasing significantly in early infancy and then steadily declining to near adult values by 3 years of age. Therefore, lipophilic drugs often have a higher volume of distribution in infants and toddlers compared with neonates, older children and adults [14].

Developmental changes in protein binding also effect drug distribution in the neonate. Neonates and young infants have decreased total plasma proteins, which may increase the free fraction of highly protein bound drug available for therapeutic effect. In neonates, presence of fetal albumin, which has reduced drug binding affinity, and increased bilirubin and free fatty acids may contribute by displacing bound drugs from the plasma protein [12]. (See Chapters 81-84 for further details.)

52.2.3 Metabolism

Metabolism and elimination determine the clearance of a given drug. Patients who have delayed clearance are at risk for drug accumulation and toxicity, and often require changes in dose or dosing interval. Developmental maturation of metabolizing enzymes occurs in both the phase I cytochrome P450 enzymes as well as the phase II conjugation reaction enzymes. For the cytochrome P450 enzymes, this activity matures in a predictable but heterogeneous pattern throughout infancy and childhood. While specific enzyme activity varies, in general the activity of CYP enzymes is decreased at birth and gradually increases over the first 3 months of life [19]. The non-oxidative metabolic reactions of methylation, acetylation, sulfation, glucuronidation, conjugation with amino

acids (primarily with glycine), glutathione conjugation and esterase hydrolysis function to detoxify and enhance the elimination of drugs and pro-drugs, many as phase II reactions subsequent to oxidation. Maturation of phase II conjugation enzyme activity occurs at a much less predictable pace [19]. These developmental changes in metabolism are likely to contribute to the unique toxicities seen in infants and children.

52.2.4 Elimination

Unlike the often irregular maturation of drug metabolizing enzyme activity, development of renal function is often predictable and directly applied in selection of drug dose and interval [19]. Premature infants have fewer glomeruli than term neonates. At birth, the term neonate has the same number of glomeruli as the adult, but the tubular system is less developed [13]. The glomerular filtration rate (GFR) is reduced in the neonate due to decreased renal blood flow and immature tubular structures [13]. The GFR in the term neonate is approximately 2 to 4 mL per minute per 1.73 m^2 [12]. Premature infants have an even lower GFR than the term newborn, with values as low as 0.6 to 0.8 mL per minute per 1.73 m^2 [12]. The GFR steadily increases to adult values by 5 to 12 months of age [12, 14]. Tubular secretion is also reduced at birth and steadily increases to adult values around 7 months of age [14]. Recent research in animal models suggests that ontogeny of renal drug transporters such as P-glycoprotein may also play a role in clearance of renally excreted drugs [20]. Failure to account for these developmental changes in clearance may lead to increased drug accumulation and toxicity.

52.3 Protein Binding

As mentioned above, protein binding plays a significant role in drug pharmacokinetics and can pose a unique challenge in the neonate. In plasma, a given drug is present in both unbound and bound states. The portion of a drug which is unbound to plasma proteins is described as the free fraction of the drug. It represents the portion of drug available for pharmacologic effect as well as clearance and elimination. The ratio of free to bound concentrations is unique to each drug, but can be influenced by physiologic and developmental factors. Highly protein bound drugs are confined to the vascular compartment and are considered to have a low volume of distribution. Drugs that are primarily unbound widely distribute to tissue, and have a large volume of distribution. Changes in total protein may alter the volume of distribution of a drug, requiring dosing adjustments to achieve optimal therapeutic effect. Free drug is usually eliminated through hepatic metabolism or renal excretion. Highly protein bound drugs may have delayed clearance and elimination, thereby prolonging the effect of the drug. Drug interactions may occur when drugs compete for plasma protein binding sites or when one drug displaces another drug from a binding site. Alterations in the free fraction of drug due to changes in total protein or drug interactions may lead to diminished therapeutic effect or toxicity.

Protein binding and drug distribution are influenced by age related changes in body composition [12]. Neonates have lower total protein levels (including albumin and alpha 1-acid glycoprotein) compared to older infants and children, which may decrease the bound portion of a drug. Fetal albumin also has a lower affinity for drugs, and the presence of endogenous substances such as bilirubin and free fatty acids compete for protein binding sites [15]. For highly protein bound drugs, these factors may contribute to an increase in the free drug fraction despite a normal total concentration (bound plus unbound), which may result in a greater pharmacologic drug effect or toxicity [15]. Examples of drugs that may be influenced by protein binding in the neonate include: phenytoin, salicylates, ampicillin, naficillin, sulfisoxazole, sulfamethoxyphrazine, furosemide, and phenobarbital [12]. These drugs tend to have an increased free fraction in the neonate, with a larger volume of distribution and may cause drug displacement or hyperbilirubinemia. In neonates, developmental changes in plasma proteins must be considered to achieve the optimal therapeutic pharmacologic effect.

52.4 Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) is the practice of sampling drug concentrations in body fluids to optimize therapeutic effects and decrease toxicities by application of pharmacokinetics and pharmacodynamics [21]. Indications for TDM include suspected drug toxicity, subtherapeutic response to drug therapy, assessment of drug interactions, assessment of clinically unstable patients, or after initiating new drugs or changing the drug regimen [22]. Therapeutic drug monitoring can guide therapy for patients with previous toxicity or adverse drug reactions to a prescribed agent [23]. Many drugs are subject to interindividual variability, and therefore, the usefulness of TDM for these drugs has to be assessed individually [24]. Agents requiring TDM have a narrow therapeutic index, a high incidence of adverse effects, or are associated with clinically significant interactions [25]. Therapeutic drug monitoring is clinically useful for agents that have a known relationship between measured body fluid concentration and therapeutic effect. Neonates, with rapid developmental changes occurring in the handling of drugs and drug response [12, 19, 26], benefit from TDM for commonly prescribed medications such as aminoglycosides and vancomycin [27, 28]. Optimization of aminoglycoside serum concentrations to achieve a desired therapeutic range warrants therapeutic drug monitoring to achieve efficacy and avoid toxicity [29, 30]. Aminoglycoside efficacy is related to the ratio of peak serum concentration with the minimal inhibitory concentration (MIC) of the infecting microorganism and area under the time-versus-concentration curve (AUC) [31]. Peak minimal inhibitory concentration ratio

of >10 are considered to be essential, therefore adequate initial peak serum concentrations of 5 to 10 mg/L are targeted by TDM [32]. Toxicity has been related to high predose ("trough") serum concentrations, therefore trough target ranges are 0.5 to 2 mg/L. Large interindividual differences occur in pharmacokinetic parameters secondary to varying levels of gestation, concomitant medications and predictive performance of initial empiric regimens might be improved by TDM.

Traditionally, TDM sampling for aminoglycosides has been performed at steady-state, around the fourth dose. This may not be practical in neonates during the first week of life. Antimicrobial therapy in neonates is often "presumptive" and may be discontinued after 72 hours of negative blood cultures. Because of prolonged half-life and reduced clearance in premature infants, intervals of 24 to 48 hours are commonly recommended [33]. Many centers are using extended interval dosing of aminoglycosides for neonates typically 4–5 mg/kg per dose administered at intervals of 24 hours or longer compared with conventional dosing of 2–3 mg/kg per dose administered at 8 to 24 hours [28]. Steady state TDM in the neonatal intensive care unit would not be performed in time to be of practical use.

Vancomycin continues to be used extensively for newborns in neonatal intensive care units for treatment of gram-positive infections [34, 35]. Initial vancomycin dosage regimens are based on creatinine clearance, weight, and postconceptional age [11]. Vancomycin dosing guidelines often require clinician interpretation using infant specific parameters to attain serum trough ranges multifold the MIC [33, 36]. Peak serum vancomycin concentrations have not been consistently associated with toxicity or efficacy in neonates; therefore routine sampling of peak serum concentrations may not be necessary. The inadequacy of current vancomycin dosing guidelines for achieving therapeutic serum concentrations using empiric dosing regimens underscores importance of appropriate therapeutic drug monitoring in neonates. Rapid changes in physiology during the perinatal period accounts for wide variability in elimination and volume of distribution for many drugs, including vancomycin. The neonatal population is heterogeneous with respect to gestational age and weight. The large variability in pharmacokinetic variables observed in this population is possibly related to factors affecting volume of distribution such as administration of ante- and postnatal medications, volume expanders, comorbidities, renal function and protein binding. A challenge for clinicians is to individualize current vancomycin dosing for very low birth weight premature neonates and critically ill term infants. While pharmacokinetic parameters for vancomycin have been characterized in newborns of varying maturity, utilization of contemporary dosing guidelines frequently results in serum concentrations outside of desired therapeutic range. Peak serum vancomycin concentrations of 25 to 40 mcg/mL and 5 to 10 mcg/mL for troughs are generally accepted as therapeutic. However, the MIC's for vancomycin have been increasing [37]. Vancomycin serum trough concentrations greater 10 mcg/mL may be indicated for improved outcomes. The requirement for achieving aggressive target vancomycin concentrations is prompting re-evaluation of the current dosing guidelines.

Prospective dosing regimens are needed for targeting higher troughs in view of emerging resistance patterns. Further assessment of physiologic factors affecting variability is warranted. Individualization of dosing may result in less serum concentration sampling and decreased time to achieving desired steady state decreased serum concentrations, thereby minimizing risk and improving outcomes for these fragile infants.

52.5 Age Specific Drug Dosing

Drug dosing for children is often derived from adult dosing regimens. Using factors such as age, weight and body surface area to extrapolate from adult doses assumes a linear relationship between development and growth. None of these methods correlate well with pharmacokinetic parameters such as drug distribution. Body surface area correlates better than weight or age for certain parameters including cardiac output, extracellular fluid, blood volume, respiratory metabolism and GFR [14, 38]. In general, weight based dosing often results in the under-dosing of infants and children, while overdosing neonates. Medication dosing using body surface area often overestimates the dose for infants [38]. Ideal dosing regimens are based on pharmacokinetic and pharmacodynamic variables from studies in neonates, toddlers, children and adolescents.

Drug dosing in children is further limited by appropriate dosing formulations. Lack of a liquid formulation may present an administration barrier for infants and young children unable to swallow tablets or capsules. Tablets, which are crushed, or capsules, which are opened, often lack pharmacokinetic data on absorption and may be irritating to the stomach [11]. Suspensions, such as phenytoin, may deliver variable drug doses if the particulates settle and are not adequately shaken before administration. It is important to remember that bioavailability is often formulation dependent. For example, phenytoin suspension is free phenytoin acid, while phenytoin capsules are phenytoin sodium, which contains only 92% phenytoin. Switching between formulations without adjusting for differences in bioavailability may result in toxicity. Clinicians should consult with a pharmacist for bioavailability data and extemporaneous formulations that may be prepared for new medications [39].

52.6 Conclusions

Safe and effective use of medications during the neonatal period is challenging due to developmental changes in physiologic and biochemical processes, which govern pharmacokinetic and pharmacodynamic responses. Developmental

variations in absorption, distribution, metabolism and elimination influence the neonate's ability to handle a drug, must be considered when selecting a therapeutic regimen. The neonate's low total protein level and decreased affinity at protein binding sites further effect drug behavior, and may lead to subtherapeutic or supratherapeutic drug concentrations. Therapeutic drug monitoring is essential in achieving and maintaining optimal therapy in neonates, however pharmacokinetic parameters, post-conceptional age and neonatal weight must be taken into account. Ideally, drug dosing in a neonate is best based upon drug specific pharmacokinetic and pharmacodynamic parameters. However, many medications lack the rigorous testing required in neonates, and further research is needed. Infants of differing gestational ages and weights must continue to be enrolled in drug trials with appropriate safety and efficacy outcomes. Clinicians are urged to consult current literature and share their experience with new agents in this vulnerable population.

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Infants of Drug-Addicted Mothers

Eunji Kim and Gideon Koren

53.1 Introduction

The wide variety of addictive substances share in common the reinforcing feature of individuals experiencing a persistent desire to administer the drugs and a strong obsession in obtaining them. Addiction to psychoactive substances is a complex issue, recognized as a class of mental disorders in the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [1]. DSM-IV defines addiction as manifested by the compulsion to take a drug with a loss of control on the intake, and continued use despite knowledge of adverse physical and psychological health effects.

The harm associated with substance use becomes increasingly alarming when women are addicted to drugs during pregnancy and mothering. The incidence of maternal substance use is difficult to assess with an estimate of 4% of women using illicit drugs and 12% of women using alcohol in pregnancy [2, 3]. The effects of maternal substance use on human development and growth are further exacerbated by behavioral and environmental factors surrounding the addiction.

Although society has a negative perception of women using recreational drugs during pregnancy, many addicted women are highly motivated and interested in the well-being of their unborn child. Reports indicate that a significant number of women seek treatment and support programs, or reduce their drug use upon discovering their pregnancies [4, 5]. Therefore, the child-bearing time offers a unique window of opportunity in engaging these women and addressing the concerns associated with their alcohol and substance use.

An understanding of the effects of perinatal drug consumption and the associated risks to the developing child is critical in providing an appropriate care to these women.

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53.2 Alcohol

Alcohol is a mild CNS depressant with potential for abuse due to its reinforcing properties. In developed countries, approximately 1 in 12 women develop alcohol dependence [6], and the potential adverse effects of perinatal alcohol consumption cannot be overstated.

Alcohol readily crosses the placenta and enters the fetal circulation with a slower elimination rate than from the maternal circulation [7]. The teratogenic effects of alcohol have been widely documented with several suspected mechanisms of action [8–11].

Fetal alcohol syndrome (FAS) describes the full-blown symptoms following prenatal exposure to alcohol [12]. The occurrence of FAS is in 0.5 to 2 per 1000 live births, and may reach as high as 10% in some communities where problem alcohol consumption is more prevalent [13]. Although variability in the characteristic features of FAS exists among affected infants and children, the consistency of the characteristics has been generally reliable. The diagnosis of FAS includes confirmation of alcohol exposure in utero along with three major criteria [14, 15]:

1. Prenatal and postnatal growth restriction
   - slow growth, when corrected for other factors, such as age, nutritional status, pathology and genetic factors.
2. Craniofacial malformations
   - midface hypoplasia
   - short palpebral fissures
   - hypoplastic filtrum
   - thin upper lip
   - epicanthal folds
   - short upturned nose
   - wide depressed nasal bridge.
3. CNS abnormalities
   - microcephaly
   - learning disabilities
   - fine or gross motor problem
   - poor executive functioning and abstract reasoning
   - pervasive behavioral anomalies.

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

The growing understanding of the use of alcohol in pregnancy has led to the realization that FAS represents only a small portion of offspring exposed to heavy alcohol in utero. In fact, many individuals affected by prenatal exposure to alcohol do not display the classical features of FAS.

Fetal alcohol spectrum disorder (FASD) is a comprehensive terminology, which recognizes the broad range of symptoms exhibited by an individual injured by maternal drinking in pregnancy. Alcohol related birth defect is used to describe various physical malformations associated with prenatal exposure to alcohol, whereas alcohol-related neurodevelopmental disorder characterizes the specific neurobehavioral and cognitive impairments of FASD.

The lack of the physical symptoms may present difficulties in clinical identification of FASD. Studies suggest that 30 to 50% of children born to problem drinkers may exhibit the characteristic features of FASD [16]. The number is concerning since FASD is the leading preventable cause of mental retardation and neurobehavioral deficits in the developing world [17]. In addition to malformation, a quarter of newborns, whose mothers were intoxicated during delivery, may exhibit ventricular tachycardia, tremors, and in some cases seizure [18].

Although diagnosing an individual with FASD may lead to labeling and stigmatization, the importance of early identification must be recognized. The damage following prenatal exposure to alcohol persists into adolescence and throughout adulthood. The term secondary disabilities of FASD identifies the disruptive school experience, mental illness, drug issues, delinquency, inappropriate sexual behavior, dependent living, and unemployment, which result from the poor social adaptability and problematic behaviors in an FASD individual [19, 20]. Early diagnosis and intervention in childhood (before age 6) were determined to be critical factors in the prevention of the secondary disabilities.

Alcohol is a known teratogen, which results in significant, lifelong deficits in physical growth, psychological health and cognitive functioning of an individual. Due to the combination of lack of knowledge on a safe amount of alcohol in pregnancy, and the absence of specific treatment for FASD, women who are pregnant or planning pregnancy should refrain from alcohol consumption. Identification of women with problem drinking in the primary care setting is an important factor in the prevention and reduction of injury caused by alcohol exposure in utero. The screening includes asking all women of reproductive age on their use of alcohol as part of routine health examination, and adopting a validated screening tool such as TWEAK (Tolerance Worry Eye opening Amnesia Cut-down) or T-ACE (Tolerance Annoyed Cut-down Eyeopener) to further screen for women who may be at risk for alcohol dependence [21].

Women who are identified with problem alcohol consumption should be encouraged to discontinue their alcohol use since stopping at any time during pregnancy can improve child health. Furthermore, they should be referred to appropriate support and treatment programs to ensure a continuum of care. In order to facilitate the early identification and intervention of infants who may be affected by alcohol exposure in utero, the frequency and the amount of maternal alcohol consumption should be recorded in the child's health documents [21].

53.3 Cannabis

The dried leaves and flowering parts of the hemp plant are smoked recreationally, constituting the most commonly used illicit drug worldwide [22]. Cannabis plants contain numerous active agents with the primary psychoactive ingredient, the delta-9-tetrahydrocannabinol (THC). Estimates of marijuana smoking in pregnant women vary between 2% to 20% [23, 24].

Although THC crosses the placenta and reaches the fetus, marijuana has not been identified as a human teratogen [25–27]. A dose-dependent reduction in prenatal growth has been reported. The suggested mechanism is the increase in carboxyhemoglobin in marijuana smokers, which may impair fetal oxygenation [26]. This may be accompanied by a reduction in placental blood flow resulting from the increase in maternal heart rate and blood pressure. However, the impact of marijuana on fetal growth has shown weak association, and a meta-analysis could not replicate the data when the possible effects of cigarette smoking were controlled for [28].

Studies on the postnatal health of children exposed to marijuana in utero have been largely inconsistent. In general, prenatal marijuana exposure did not affect the offspring's cognitive abilities [29–31]. Subtle effects have been reported, which included abnormal sleep pattern in infancy, poor visual responsiveness and sustained attention. However, many of the studies recognized the possible confounding role of postnatal home environment of these children.

53.4 Cocaine

Cocaine is a potent, short-acting stimulant, which can be snorted or injected. The derivative form, known as crack, is smoked. Crack and cocaine are the second most common illicit drugs used by pregnant women. Cocaine freely crosses the placenta, and may reduce uterine blood flow via its vasoconstrictive effect [32]. Although cocaine has not been unambiguously identified as a human teratogen, many studies report adverse maternal and neonatal health following cocaine exposure. Cocaine use during pregnancy has been associated with an increased risk for intrauterine death, pregnancy complications such as placenta abruption, premature rupture of membranes and preterm birth [33–36]. Maternal dependence of cocaine in pregnancy has been associated to increase the risk for low birth weight and a small head

circumference [37, 38]. The use of cocaine near term may result in transient neurologic abnormalities of newborn including disrupted sleep, irritability, tremor and hypertonia [39].

Numerous neurodevelopmental studies conducted on children prenatally exposed to cocaine have largely resulted in conflicting results. The prevailing consensus is that maternal use of cocaine in pregnancy is not associated with global cognitive deficits [38–41].

Subtle effects such as language delays or behavior problems have been described. The inconsistent outcome of children exposed to cocaine in utero may in part be due to the difficulties associated with studying cocaine addicted women and their children.

Moreover, many cocaine users combine other substances, such as alcohol, a known teratogen. Importantly, the general consensus is that when children of cocaine dependent mothers are provided with appropriate care and support, the long-term assessments resulted in favorable outcome with a normal pattern of development.

53.5 Other Stimulants

Amphetamines have been used therapeutically for various purposes such as narcolepsy, appetite suppression, and attention deficit hyperactivity disorder (ADHD). These drugs may be purchased illegally on the street and used for recreational purposes.

Dextroamphetamine (Dexedrine), widely prescribed for obesity in the past, can be injected intravenously for its stimulatory effects. Methylphenidate (Ritalin), the first line treatment for ADHD, can also be used as a drug of abuse by oral or intranasal ingestion.

Methamphetamines are also produced in illegal laboratories and sold as speed, ice, crank and crystal meth.

Studies on the effects of stimulants other than cocaine have resulted in mixed conclusions. Numerous case reports suggest an association between amphetamine use in pregnancy and various malformations including congenital heart abnormalities, biliary atresia and oral cleft [42–44]. However these studies are often limited by small sample size and poor methodology. Some studies report no increased risk for malformation [45, 46], but possible increased risk of premature birth and intrauterine growth restriction [45, 47]. The suggested mechanism of action is its vasoconstrictive effect, which may impair oxygen flow to the fetus. However, the appetite suppressant characteristics of amphetamine, the energy surge experienced by the users, in combination with many cofounding risk factors make a definite conclusion impossible. Babies exposed to amphetamine close to delivery may experience transient jitteriness, drowsiness, and respiratory distress [47].

The neurotoxicity of amphetamine on a developing brain is currently unknown. The limited human data have reported reduced brain structure volume, poor performances on sustained attention, and verbal memory deficits [48]. However, the direct effect of methamphetamine exposure in utero and the postnatal home environment are difficult to differentiate.

In addition, children who live in methamphetamine home laboratories face health and safety hazards from explosions during manufacturing, and airborne exposure to toxic chemicals. The environments of amphetamine users are often characterized by violence, chaos, neglect, abuse, and criminal behavior [49].

53.6 Opioids

Reports indicate that the non-medical use of opioids is growing with current estimate of 2% of pregnant women users [50]. Opioid such as codeine, morphine, meperidine, oxycodone, hydrocodone, fentanyl and heroin can be ingested orally, injected intravenously or intramuscularly, snorted or smoked.

Although opioids do not increase the risk for birth defect, an increased incidence of obstetric complications in opioid dependent women has been reported with high rate of preterm birth and intrauterine growth restriction has been suggested [51]. It has been argued that the effects of opioids on growth may be related to confounding variables such as poor maternal nutrition and concurrent drug use.

The maternal use of opioids near term can result in neonatal abstinence syndrome with a reported incidence in 60-80% of infants born to opioid dependent women [52]. Withdrawal typically begins within 24 hours, but symptoms can be delayed for up to two weeks.

Clinical features include insomnia, irritability, hypertonia, tremor, vomiting, diarrhea, fever, high pitch crying, and seizures.

Studies have suggested poor developmental outcomes following prenatal exposure to opioid, which include sleep disturbances, short attention span and hyperactivity [53, 54]. The authors recognized the difficulty in separating cofounding factors such as maternal polydrug use and postnatal home environment from direct effect of maternal opioid use in pregnancy. Detoxification during pregnancy should be avoided as sudden withdrawal of the mother has been associated with adverse maternal, fetal and neonatal health.

Maternal symptoms of withdrawal include abdominal cramping and uterine irritability, which may increase the risk of miscarriage, preterm labor, and fetal death [55]. Therefore, if detoxification is to be undertaken during pregnancy, it should involve a slow tapering of the opioid under the supervision of trained health care professionals. Women should also be counseled on methadone or buprenorphine treatments, which have been reported to improve maternal and neonatal health.

53.7 Sedatives-Hypnotics

53.7.1 Barbiturates

Although the use of barbiturates has decreased dramatically over the last years, barbiturates are still prescribed for the treatment of seizure disorder unresponsive to standard therapy, and are included in headache preparations. Widely used barbiturates include mephobarbital, pentobarbital, phenobarbital and secobarbital. Barbiturates may be used recreationally for their intoxicating effects. Heavy stimulant drug users may use barbiturates to counteract the effects of large doses of amphetamines or cocaine. Early data suggested teratogenicity following maternal barbiturates use in human pregnancy.

However, results were confounded by the severity of maternal epilepsy and polytherapy [56]. More recent data on monotherapy does not suggest teratogenicity [57]. It is important to keep in mind that women were using barbiturates for therapeutic reasons, and positive findings may not apply to frequent use at high dosage in drug-addicted women. Infants born to mothers receiving barbiturates near term may show transient signs of restlessness, sleep disturbances, and respiratory depression [58]. These symptoms may be delayed up to two weeks postpartum due to the long half-life of barbiturates.

53.7.2 Benzodiazepines

Benzodiazepines have a wide clinical use ranging from treatment for anxiety, insomnia, and as muscle relaxants and anticonvulsants. Commonly used benzodiazepines include chlordiazepoxide, diazepam, lorazepam, and oxazepam. Although benzodiazepines in pregnancy have been suggested to increase the risk for oral cleft [59], a recent study on 1979 infants did not find an increased risk [60]. Combination of benzodiazepine with other substances and use at high dosage may result in distinctive neonatal outcome than that observed at therapeutic dosage. Maternal use of benzodiazepine near term has been associated with transient lethargy, poor muscle tone, and respiratory depression in neonates.

53.8 Club Drugs

Club drugs include a variety of psychoactive compounds, which are commonly used at nightclubs, bars, raves, or trance scenes. Ecstasy (N-methyl-3,4-methylenedioxyamphetamine, MDMA), gamma-hydroxybutyrate (GHB), ketamine, lysergic acid diethylamide (LSD), flunitrazepam (Rohypnol) are common examples.

MDMA belongs to the amphetamine family. Although isolated case reports suggest a possible increased risk for congenital heart malformations, small sample size, an inconsistent pattern of heart malformations, and poor methodology make it difficult to estimate the risk, if it exists [61].

GHB, a metabolite of the inhibitory neurotransmitter gamma-aminobutyric acid, is used for the treatment of narcolepsy and as a general anesthetic. It is also used as recreational drug due to its euphoric and sexually enhancing effects. A woman addicted to GHB delivered a healthy full-term birth followed by a mild maternal respiratory depression [62]. However, large studies are needed to identify perinatal risks of GHB use in pregnancy.

Ketamine, a dissociative anesthetic, has not been studied for its potential human teratogenic effect, although animal data do not suggest an increased risk for malformation [63]. All existing human data resulted from its use as an epidural for pain relief during labor [64].

A higher incidence of limb and eye defects has been described in isolated reports of LSD addiction in pregnancy [65], although larger prospective studies could not replicate these findings [66]. Possible increased risk of spontaneous abortions has been suggested, but more studies are needed before a definite conclusion can be drawn.

In general, club drug users have different pattern of use compared to other substance users with different risk factors. They are more likely to be teenagers or young women with a combination of different drugs, most commonly alcohol. The lifestyle factors associated with clubs and raves may also contribute to poor outcome of infants and children of these women.

53.9 Solvents and Inhalants

Solvents and inhalants are not commonly used by pregnant women. However, women using inhalants are often at a social disadvantage with the inability to obtain other substances. Solvents and inhalants contain gasoline or toluene, which are pharmacologically quite similar to alcohol. The reported effects on pregnancy include preterm birth, intrauterine growth restriction, microcephaly, neonatal electrolyte disturbances (hypobicarbonatemia or hypokalemia) and developmental delay [67]. There are reports of children with facial features similar to FAS, which was coined as toluene embryopathy.

53.10 Comorbidities

Drug addiction is a complex medical condition with many risk factors surrounding drug abuse. Studies on offspring of addicted women recognize the difficulty to disentangle the effects of numerous comorbidities from the direct damage of substance use on a developing child.

The postnatal period may in fact be the most critical point of a child's development.

Some of the challenges associated with maternal substance use include [68–70]:

- lack of prenatal/postnatal care and available resources
  - poor nutrition
  - inadequate housing
  - lack of access to health care
  - lack of education and employment skills
- low socioeconomic status
- lack of family and partner's support
  - disruptive family experience
- maternal ineptness
  - inadequate bonding and nurturing
  - lack of appropriate stimulation
  - inappropriate role model
- psychiatric disorders or emotional instability
- transmission of sexually transmitted disease
- history of violence, abuse and neglect
- genetic predisposition.

These factors have all been associated with impaired intellectual abilities and social behaviors of the children, and long-term outcomes improved significantly when women and their children were provided with prenatal and postnatal intervention services.

Maternal alcohol and illicit drug use in the perinatal time frame has been associated with physical and neurodevelopmental abnormalities in the offspring. Polydrug use and the risk factors surrounding substance use make it difficult to discern the specific impacts of a drug on the developing child. However, the consistent reports of adverse outcome of children born to addicted mothers emphasize the need to address the associated issues.

To reduce harm incurred by maternal substance use, it is important to routinely screen all women of reproductive age for their consumption of alcohol and illicit drugs. When women's problem alcohol and drug use is confirmed, it is important to engage and motivate them to change their risky behaviors. Furthermore, they should be referred to appropriate treatment and support services to assist them in their challenges. Programs may include shelters, employment resources, food banks, and childcare services.

Biological samples of the newborn, such as meconium and hair, is often requested in infants with suspected but unconfirmed exposure to alcohol and illicit drugs.

In case of significant level of exposures, women should be counseled on the benefits of discontinuing at any time. Their infants should be monitored routinely by a pediatrician and provided with facilitated access to support services to match the needs of the infant.

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Infants of Smoking Mothers

Roberto Paludetto and Francesco Raimondi

54.1 Introduction

Knowledge of the adverse effects of tobacco products on the health of the adult has long gained acceptance. Scientists and clinicians are now unraveling the multifaceted relationship between tobacco smoke and human development starting from the intrauterine and possibly periconceptional phase. Evidence now correlates maternal smoke to derangement of organogenesis, decreased organ function and poorer neurodevelopmental performance. However, findings are not always unequivocal mainly because of methodological problems. In this chapter, we will critically address some of the current issues focusing on the available clinical evidence to give the reader a practical approach to the infant of the smoking mother.

54.2 Epidemiology

International authorities monitoring the smoking habits and their consequences consider tobacco as a "legalized health hazard" on a planetary scale. In 2007, the WHO classified 18.2% of European adult females as smokers [1]. Figures vary among individual countries but have remained substantially stable since the previous report in 2002. Separate data for smoking during pregnancy are not available on this scale and have to be deduced from epidemiological surveys. Reports from Sweden and the US estimate respectively that 13% and 10.4% of women smoke during their pregnancy, but more recent work from the UK has come up with a figure as high as 36% [2]. The percentage of women who smoke around the time of conception is higher as most women who stop smoking achieve this goal very early during the pregnancy. However, since many will attempt to quit smoking throughout their pregnancy, it is mandatory that they receive constant support and encouragement by healthcare professionals. This should continue after delivery, since many women who quit smoking during pregnancy will resume this habit shortly after giving birth. Moreover, Cnattingius et al have recently demonstrated that a previous adverse pregnancy outcome has only a modest influence on smoking habits in a successive pregnancy [3].

Besides direct smoking, a pregnant woman and her fetus may be exposed to environmental tobacco smoke (ETS). While the detrimental effects of this condition will be discussed below, it is difficult to gather good quality data on its prevalence. In 2007, Ward et al reported that in the UK as many as 13% of infants were born to non-smoking mothers who had had significant exposure to ETS [2]. Maternal smoking during pregnancy and ETS may have additive adverse effects on infantile health.

54.3 Pharmacology of Tobacco Smoke and its Detection in Biological Specimens

Tobacco smoke has many compounds, some of which might have effects on an infant's health that have not been yet investigated.

Carbon monoxide rapidly binds to both maternal and fetal hemoglobin with carboxyhemoglobin levels that eventually become higher in the fetus than in the mother because the former has a higher hemoglobin affinity for carbon monoxide and a longer elimination half-life than the latter. This leads to a potentially profound impairment of oxygen delivery to the tissues that can be aggravated by other smoke-derived chemicals (thiocyanates and other cyanides) that also bind hemoglobin.

Nicotine is the chemical that is primarily responsible of tobacco addiction and organ toxicity. In the brain, nicotine binds to nicotinic acetylcholine receptors eliciting the release of many neurotransmitters. Nicotine can also act peripherally

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

stimulating the release of epinephrine, ultimately increasing heart and respiratory rate and causing vasoconstriction. It also suppresses insulin release possibly affecting glucose blood levels and appetite. Nicotine passes into breast milk with concentrations that are in linear correlation with its levels in maternal serum [4].

Cotinine, the major nicotine metabolite, is currently used to objectively assess an infant's exposure to tobacco smoke both before and after birth. Cotinine may be dosed in plasma, urine, saliva, hair and meconium. This sampling is expensive, may be invasive (i.e., plasma) and depends on cotinine half-life (except for hair) but often reveals a greater exposure to tobacco smoke than maternal self-reports. In the study by Puig et al, infantile exposure to ETS was significantly higher when assessed by infant urinary cotinine compared to parental self-reported [5]. Similarly, 64.3% pregnant women who claimed to be non-smokers and not exposed to ETS had plasma cotinine levels above the limit of detection [6]. Despite the proven value of this biomarker, much of the research published to date still relies on self-reports.

54.4 Smoke, the Pregnancy and the Fetus

Nicotine is a powerful vasoconstrictor that can significantly reduce maternal blood supply to the fetus. The fetal blood supply is further impaired by placental alterations and high carboxyhemoglobin levels. These effects combined may explain why smoke during pregnancy has been associated with perinatal death, spontaneous abortion, stillbirth, abruptio placentae, placenta previa, fetal growth restriction, preterm birth and low birth weight. In a recent cohort study on 7098 pregnant women, the association with low birth weight was particularly true for active smoking during late pregnancy, while quitting smoking early in pregnancy was associated with a higher birth weight than continuing to smoke. A slight, non-significant trend to a beneficial effect on birth weight has been described for reducing the number of cigarettes without quitting completely [7]. Infant length and head circumference are also affected though probably to a lesser degree. Kannellopoulos reports that tobacco-related effects on fetal growth may be reversible during the first few years of life [8].

54.5 Neonatal Consequences of Smoke Exposure

The multifaceted damage of smoking during pregnancy on neonatal health starts with an increased rate of congenital malformations. Many questionnaire-based studies have reported a greater incidence of cleft lip and palate in infants of smoking mothers [9]. This piece of data has been recently confirmed by a biomarker-based study. Women with a serum cotinine above 2 ng/mL have a 2.4 odds ratio for delivery of a child affected by an orofacial cleft [10].

Congenital heart defects have also been associated to prenatal smoke exposure. Recently, a large questionnaire-based, case-control study conducted by Malik et al concluded that women who smoked around the periconceptional period were more likely to give birth to neonates with septal and right-sided obstructive defects [11].

Impaired development of the fetal femur and/or of its vasculature has been claimed to explain the increased risk of Legg-Calvè-Perthes disease in the offspring of mothers who smoked during pregnancy compared with matched controls [12]. More research is required to re-evaluate this topic using biomarkers as an objective measure of the infant's smoke exposure.

Besides organogenesis, neonatal organ function is impaired by direct maternal smoking during pregnancy or by ETS.

Studies of pulmonary mechanics in infants with prenatal exposure to tobacco smoke have shown an alteration in expiratory flow profile, a reduction in respiratory compliance and an increase in airways resistance [13].

Prenatal smoke exposure has a long lasting effect on expiratory flow rates as a significant reduction has been reported by Cunningham et al [14] in 8–12 year old children. The respiratory function deficit was larger for black children than for white and for boys than for girls [15]. The severity of wheezing may be further influenced by beta-2 adrenergic receptor variants in a complex gene-environment interaction [16].

Children of mothers who smoked during pregnancy have a higher risk of respiratory tract infections both during the neonatal period and during the first year of life. The same holds true, to a lesser degree, for ETS, which could directly damage the respiratory epithelial lining and also suppress the innate immune system [17].

A large, questionnaire-based, cohort study has recently evaluated the effects of maternal smoking in pregnancy on the cardiovascular function of the neonate [18]. Infants of smoking mothers had a 5.4 mmHg higher systolic pressure than offspring of non-smoking mothers but no significant association was found with diastolic pressure or heart rate. ETS alone (i.e., infants of non-smoking mothers but with smoke exposure in the household) did not have modified blood pressure or heart rate compared to controls.

54.6 Behavioral Aspects of the Offspring of the Smoking Mother

The profound impact of nicotine on neural cells suggests a possible effect on neonatal behavior. Questionnaire-based research results, however, have not been entirely clear. Most studies examined tobacco in the context of other factors such as illicit substance abuse or obstetric complications [19]. Our group has conducted a prospective analysis of neonatal behavior, assessed

by the Brazelton Neonatal Behavioral Assessment Scale, monitoring the infant's urine cotinine and administering a structured questionnaire to the parents [20]. Newborns of mothers who smoked during pregnancy had a higher urinary cotinine excretion. They were more irritable and less interactive with the environment when compared with controls. The same was true, although to a lesser extent, for offspring of non-smoking mothers but smoking fathers, used as a proxy of ETS. Using a different evaluation tool, Stroud et al came to similar conclusions [21]. Neonates of smoking mothers had an increased arousal and excitability throughout the first month of life, well past nicotine half-life. This has to be taken into account to prevent early disruption of a correct maternal-infant bonding.

The adverse behavioral effects of prenatal smoke exposure extend far beyond the neonatal period. Schmitz et al have documented an odds ratio of 3.44 for an increase of the inattentive form of attention deficit hyperactivity disorder (ADHD) and some studies show a higher rate of smokers among adolescents affected by ADHD as they were trying to self-medicate to alleviate cognitive or attentive disorders [22]. A prospective longitudinal study by Hook et al has linked prenatal smoke exposure to externalizing problems and aggression leading to a higher incidence of delinquent behavior both in males and females [23].

54.7 Smoke and SIDS

After the recognition that sleeping in the supine position is associated with a decreased incidence of sudden infant death syndrome, maternal smoke has become the main, preventable risk factor for SIDS. There is evidence that infants of smokers are less rousable both in REM and in non-REM phase of sleep [24] and have deficient hypoxic awakening response [25]. Work in lambs exposed in utero to nicotine shows delayed arousal and reduced ventilatory and heart rate responses to hypoxia. Infants who died of SIDS had increased apoptosis in their brainstem nuclei compared to babies who had suddenly died for other reasons; cigarette smoke exposure was especially associated with increased apoptosis in the dorsal motor nucleus of the vagus and the arcuate nucleus [26]. These pathology observations may provide the link between tobacco smoke, a defective cardiorespiratory control and SIDS.

54.8 Reducing Damages from Prenatal Smoke Exposure or ETS

It is intuitive that enforcing regulations on banning smoke from public places and taxing tobacco products should reduce smoking rates and ETS. More focused programs are in place in many developed countries where smoking cessation services are available before or during the pregnancy offering behavioral support. There is currently an open debate on the safety and efficacy of nicotine replacement therapy in pregnant women [27, 28].

54.9 Conclusions

Perinatal health professionals need to increase their awareness about the detrimental effects of smoking during pregnancy. Further research conducted with reliable biomarkers of smoke exposure will improve the quality of information both about the effects of direct maternal smoke and its role as an environmental pollutant. This information is much needed if we are to design successful interventions.

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Infants of Diabetic Mothers

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55.1 Overview

While advances in maternal and neonatal medical care continue to improve the outcomes for infants born to mothers with glucose intolerance during pregnancy, the risks for spontaneous abortion, stillbirth, congenital malformations, and perinatal mortality and morbidity still exist. Abnormal maternal glycemic control caused by gestational diabetes mellitus or pregestational diabetes mellitus complicate up to 10% of pregnancies, and as the significantly overweight pediatric population develops into their child-bearing years, this number is likely to rise [1]. Pregnancies of mothers with diabetes are given increased surveillance, as the multifaceted metabolic changes that occur in the mother can place the infant at risk for periconceptional, fetal, neonatal, and long-term morbidities [1, 2]. Fortunately, appropriate periconceptional and prenatal care can improve the risks of perinatal complications by close monitoring of maternal glycemic control.

55.2 The Pederson Hypothesis and Diabetic Fetopathy

In general, maternal glycemic control remains the primary factor that determines the potential complications in the conceptus and the neonate. Some sequelae, such as failure of implantation, miscarriage and congential malformations are a function of pre and peri-conceptional maternal glucose control. Other sequelae, such as macrosomia, neonatal hypoglycemia, and fetal/neonatal iron deficiency result from worsening glycemic control in the last few weeks prior to delivery [3, 4]. Fetal hyperglycemia, hyperinsulinemia, or the combination of the two leads to the pathologic conditions seen in the late gestation fetus and neonate (Fig. 55.1).

55.3 Risks of Maternal Glucose Intolerance During the Periconceptional Period

Up to 40 known congenital malformations associated with maternal diabetes have been noted [5]. Indeed, a strong association between maternal glycemic control at the time of conception and early gestation and the incidence of congenital anomalies has been recognized in several epidemiological studies, with a significant decrease in congenital malformations reported in mothers with strict metabolic regulation during the periconceptional period [6, 7]. Anomalies of the heart, musculoskeletal system, and genitourinary system have been seen at a twofold to threefold higher incidence in infants of insulin-dependent diabetic mothers compared to the normal population [8]. The frequency of congenital anomalies is not increased in offspring of gestationally diabetic mothers [9].

The mechanisms by which these anomalies occur remain under investigation. Maternal hyperglycemia, the major teratogenic factor, may be involved in hyperglycemia-induced apoptosis that is associated with oxidative stress, lipid peroxidation, and decreased antioxidant defense capacity in the embryos [10, 11]. In studies of mouse embryos from diabetic mice, hyperglycemia appears to disturb cellular pathways specific for cardiac and neural tube development [12–14]. A three-to-fivefold increase in major malformations in infants as well as a fivefold increase in pregnancy loss rate in mothers with hemoglobin A1c levels (>7%) early in pregnancy has been demonstrated [15]. Latest recommendations include maintaining a hemoglobin A1c less than 5% in the first trimester of pregnancy and below 6% in the third trimester in healthy pregnant women with type 1 diabetes mellitus [16]. Routine screening for congenital anomalies of all infants of diabetic mothers (IDMs) is not cost-effective. The index of suspicion when evaluating a newborn IDM should clearly be very high.

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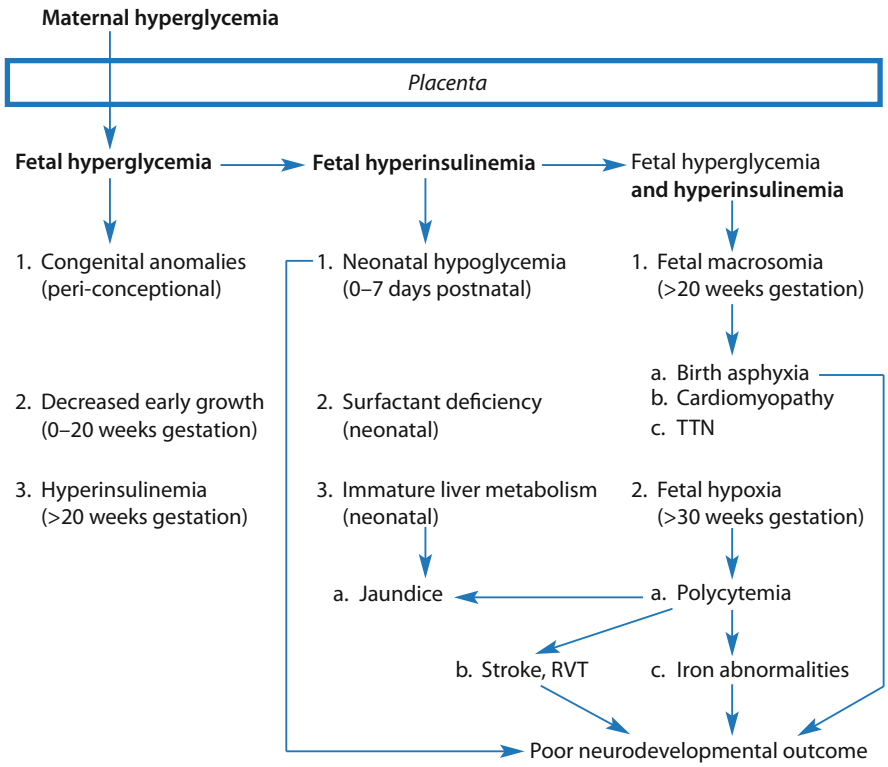

Fig. 55.1 The fetal and neonatal events attributable to maternal diabetes mellitus. Reproduced from [1], with permission

55.4 Risks of Maternal Glucose Intolerance During the Fetal Period

Maternal hyperglycemia has effects on fetal growth, glucose metabolism, oxygenation, iron metabolism, cardiac anatomy and function, and transition to extrauterine life.

55.4.1 Growth Disorders

Neonatal macrosomia is the classic presentation of an IDM and results from biochemical changes along the maternal hyperglycemia – fetal hyperinsulinemia pathway [17]. Early in the second half of pregnancy, the pancreatic islet cells mature and insulin production escalates in response to hyperglycemia creating an environment of hyperinsulinemia, a key anabolic hormone, coupled with hyperglycemia, a key anabolic fuel. The combination produces a large increase in fetal fat stores and some mild increase in protein content [1]. Hepatomegaly, splenomegaly, and cardiomegaly resulting from septal hypertrophy are often seen in the context of a macrosomic infant, while the head circumference is usually not increased. A significantly increased fetal abdominal area growth rate in the third trimester has been associated with high maternal hemoglobin A1c levels early in pregnancy [18] and has been used to effectively reduce rates of macrosomia [19]. The risk of shoulder dystocia, perhaps the most feared birth complication, is greater for an infant of any weight born to a diabetic versus a nondiabetic mother [20]. Accordingly, it has been recommended that elective cesarean be strongly considered for diabetic mothers with fetal weight estimates greater than or equal to 4250 grams.

In contrast to the pathophysiology leading to the macrosomic infant, mothers with advanced diabetic vascular disease are at risk of having fetuses with significant growth deceleration, defined as birth weight less than the fifth percentile for gestational age.

55.4.2 Disorders of Glucose Metabolism

Glucose intolerance coupled with resistance to or absence of insulin characterizes maternal diabetes during pregnancy. Although glucose crosses the placenta to the fetus, insulin that is either maternal in origin or exogenously delivered does not reach the fetus. Therefore, fetal hyperglycemia leads to pancreatic islet cell stimulation and subsequent fetal insulin production. Additionally, hypoglycemic episodes may result from sudden reductions in maternal glucose in fetuses with islet cell hyperplasia.

Both hyper- and hypoglycemia have been identified as possible mechanisms for the increased fetal mortality rate seen in diabetic pregnancies [21].

55.4.3 Reduced Fetal Oxygenation

Abnormalities in fetal oxygenation during diabetic pregnancies were discovered in the 1980's and account for the high rate of polycythemia in offspring of poorly controlled diabetic mothers [1].

The fetal hypoxia is a consequence of an elevated fetal basal metabolic rate stimulated by chronic hyperglycemia and hyperinsulinemia. With increases in rate of substrate uptake and oxidation, the fetal environment becomes hypoxic, since the higher oxygen demand cannot be met by increased oxygen transfer by the placenta (human or ovine). Several studies have demonstrated the attempt of the fetus to increase oxygen-carrying capacity in the face of hypoxemia includes elevated erythropoietin concentrations and fetal polycythemia [22–27]. Fetal hypoxia constitutes a risk to the developing brain as does the potential for hyperviscosity and fetal stroke.

55.4.4 Disorders of Fetal Iron Metabolism

Chronic fetal hypoxemia and the subsequent compensatory erythropoiesis coupled with the larger blood volume of the macrosomic fetus places a large demand on fetal iron metabolism particularly in light of limitations on maternal-fetal iron transport to support this large demand.

IDMs are at greater risk for iron deficiency of multiple organs due to the inability of the placenta to upregulate iron transport sufficiently and subsequent iron prioritization and redistribution [28–35]. These deficiencies can affect neurodevelopment into infancy and beyond [28–32]. Decreased fetal hepatic iron stores, as indexed by abnormally low cord serum ferritin concentrations, occur in 65% of live-born IDMs [26].

55.4.5 Acute and Subacute Non-Structural Cardiac Abnormalities

Fetal cardiac septal hypertrophy and cardiomegaly as a result of glycogen loading from chronic hyperglycemia and hyperinsulinemia are specific phenotype findings associated with diabetic pregnancies. Despite good maternal metabolic control, serial evaluations of cardiac growth demonstrated cardiac hypertrophy in late gestation (34 to 40 weeks) in fetuses of diabetic mothers [36] making cardiomegaly a possible contribution to the increased risk of fetal death in diabetic pregnancies [37].

55.5 Risks of Maternal Glucose Intolerance During Pregnancy on the Neonate

The abnormal metabolic milieu of hyperglycemia and hyperinsulinemia during fetal life leads to multiple issues in the neonatal period, including abnormalities of neonatal body habitus, glucose, calcium and magnesium metabolism, hematologic status, cardiorespiratory function, bilirubin metabolism, and neurologic functioning (Table 55.1).

55.5.1 Growth Disorders

Newborn macrosomia is predicted by poor maternal glycemic control, particularly in the last few weeks of gestation. Occurring in 15-45% of diabetic pregnancies, macrosomia is generally defined as birth weight above the 90th percentile for gestational age or greater than 4000 grams [38].

Macrosomia at birth serves as a marker for identifying infants at risk for neonatal morbidities, including hypoglycemia,

Table 55.1 Risk, assessment and management of neonatal metabolic complications in infants of diabetic mothers

Condition	Assessment	Action value	Management
Hypoglycemia	Serum glucose	<40 mg/dL first 24 hours; <50 mg/dL after 72 hours	Early feedings; IV D10W 2 mL/kg body weight; repeat serum glucose in ½ hour
Hypocalcemia	Serum ionized calcium	<3.5 mg/dL	Calcium gluconate or calcium chloride if symptomatic; treat hypomagnesemia prior to calcium treatment
Hypomagnesemia	Serum magnesium	<1.5 mg/dL	Magnesium sulfate
Polycythemia	Hemoglobin Hematocrit (measured centrally)	>20–23 g/dL >65–70%	Partial exchange transfusion with target hematocrit of 55%
Iron deficiency	Serum ferritin	<35 mcg/L	Follow-up hemoglobin and ferritin at 6 months
Hyperbilirubinemia	Serum indirect (unconjugated) bilirubin	Dependent on age and infant size	Phototherapy, early feedings, hydration, rarely exchange transfusion

hyperbilirubinemia, and neonatal acidosis [38]. Complete anthropometric measurements, including weight, length, and head circumference should be assessed and plotted on population-specific appropriate growth curves for each infant soon after birth.

Appropriate for gestational age IDMs have been found to have significantly greater fat mass, percent of weight that was fat, and triceps, subscapular, abdominal, flank, and thigh skinfold measurements than infants of nondiabetic women [39]. Subsequently, macrosomic IDMs generally have higher weight than length and head circumference percentiles. Infants at risk for metabolic instability in the newborn period can be better identified by body proportion measurements, such as the mid-arm-circumference-to-head-circumference ratio [40].

55.5.2 Neonatal Hypoglycemia

Significant hypoglycemia, defined as a blood glucose level less than 45 mg/dL, is seen in up to 50% of IDMs following birth, with macrosomic or growth-restricted IDMs more likely to have hypoglycemia than IDMs of appropriate size for gestational age [1, 17]. The abrupt discontinuation of maternally derived glucose combined with neonatal hyperinsulinemia leads to hypoglycemia in the macrosomic infants. Normally, this interruption in glucose delivery is followed by a decrease in neonatal plasma glucose between 30 and 90 minutes followed by spontaneous recovery in most infants [8]. Hypoglycemia seen in infants with growth restriction due to diabetic vascular disease is more likely due to decreased hepatic glycogen stores and may not appear until 6-12 hours after delivery [17]. Hypoglycemia can persist and therefore require intervention consisting of a constant intravenous dextrose infusion.

Controversy exists concerning the threshold of what constitutes hypoglycemia, as little outcome data is available for symptomatic or asymptomatic hypoglycemia. A common recommendation is that IDMs be screened at ½, 1, 1 ½, 2, 4, 8, 12, 24, 36, and 48 hours of age [17], and be treated to maintain a glucose over 40 mg/dL [41].

Although most infants with hypoglycemia are asymptomatic, typical symptoms can include jitteriness, sweating, tachypnea or apnea, seizures, agitation, and respiratory distress. Newborn IDMs, especially those who are macrosomic or growth restricted, should be monitored closely with regularly scheduled serum glucose measurements.

Maternal history, with attention to glycemic control, and fetal anthropometric measurements and proportion studies can help to identify infants at highest risk [40].

Early initiation of feedings is highly recommended, as those infants who are asymptomatic, normoglycemic, and able to tolerate feedings do not need intravenous dextrose therapy.

55.5.3 Neonatal Hypocalcemia and Hypomagnesemia

Neonatal hypocalcemia and hypomagnesemia are two frequently encountered metabolic disturbances that occur in up to 50% of IDMs during the first three days of life and are related to lack of maternal glycemic control [8]. A physiological nadir of serum calcium is seen in healthy term infants by 24–48 hours of age during the transition from fetal to neonatal parathyroid and calcitonin regulation [42]. The nadir may be exaggerated to the point of hypocalcemia, generally accepted as an ionized calcium level less than 3.5 mg/dL [17]. Ionized calcium measurements, when available, are preferable to total serum calcium measurements. Hypocalcemia in the IDM may be caused by an inadequate neonatal parathyroid response to the abrupt interruption of maternally derived calcium as well as continual high levels of calcitonin, and possibly disturbances in vitamin D metabolism [8].

Neonatal hypomagnesemia is related to maternal hypomagnesemia and severity of maternal diabetes [17]. A serum magnesium level less than 1.5 mg/dL is considered pathologic [17], and may complicate the treatment of hypocalcemia.

Similar to hypoglycemia, neonatal hypocalcemia and hypomagnesemia may present with symptoms such as jitteriness, sweating, tachypnea, irritability, and seizures. They present later than hypoglycemia with nadirs seen at 24 to 72 hours following birth [1]. Because of this similarity of symptoms, the jittery IDMs should have serum glucose, calcium and magnesium levels measured. Symptomatic hypocalcemic IDMs can be treated with calcium chloride or calcium gluconate preferably via a central venous catheter to reduce the chance of peripheral vein damage from the medication. Hypomagnesemia needs to be corrected in order to enable normalization of calcium homeostasis.

55.5.4 Neonatal Polycythemia

Polycythemia is present in 20% to 30% of infants born to poorly-controlled diabetic mothers. The definition of polycythemia is a central venous hemoglobin concentration greater than 20 g/dL or hematocrit greater than 65% [1]. Blood hyperviscosity at levels higher than these leads to morbidities including vascular sludging, ischemia, and infarction of vital organ systems [38].

The cerebral microcirculation is one area at risk for hyperviscous blood sludging, resulting in neurologic irritability, jitteriness, and a high-pitched cry. Cerebral venous sinus thrombosis must be considered in these infants, and even though neuroimaging studies may not detect pathology, symptomatic, polycythemic IDMs should be treated with partial exchange transfusion to reduce blood viscosity and the predisposition to further clot formation [1].

Symptoms of vascular sludging in the renal, intestinal, and pulmonary systems may or may not be explicit. Hypertension, thrombocytopenia, hematuria, and abdominal mass are all possible signs of renal vein thrombosis. Feeding intolerance or necrotizing enterocolitis may indicate intestinal sludging. Finally, pulmonary vascular bed sludging may present as persistent pulmonary hypertension with worsening respiratory distress syndrome.

Assessment including an initial hematocrit and platelet count ought to be obtained soon after birth. Because of the physiologic diuresis that occurs in all newborns, an increasing hematocrit over the first three days of life is not uncommon. This phenomenon, however, prolongs the risk for microvascular sludging in the polycythemic IDM, making the importance of appropriate monitoring and hydration paramount. Sludging and thrombosis of the microvasculature of any of the aforementioned organ systems may first be indicated by a declining platelet count. Asymptomatic infants with hematocrit values from 65% to 70% ought to be hydrated with intravenous fluids at a rate of at least 100 mL/kg/day, and the hematocrit should be followed daily for the first three days. If the infant becomes symptomatic or the hematocrit continues to increase despite appropriate therapy, a partial volume exchange transfusion should be completed. An immediate partial exchange transfusion is indicated in any IDM who is symptomatic from blood hyperviscosity or those with a central hematocrit greater than 70% [1].

55.5.5 Neonatal Iron Deficiency

Abnormalities of iron metabolism at birth have been demonstrated in 65% of IDMs and 95% of macrosomic IDMs [26, 43, 44]. Of note, IDMs with the highest hematocrits at birth tend to also have the lowest ferritin concentrations. Iron deficiency presents a risk to the developing brain including effects on myelination [45], brain energy metabolism [46], and brain monoamine neurotransmitter metabolism [47]. Newborn IDMs have altered cognitive processing compared to healthy, term infants [48]. Treatment with supplemental iron (beyond normal recommendations) in the immediate newborn period has not been assessed with respect to improving the neurodevelopmental outcome and is currently not recommended.

55.5.6 Pulmonary and Cardiac Status

Respiratory distress syndrome (RDS) and transient tachypnea of the newborn (retained fetal lung fluid) are more commonly seen in IDMs than age-matched controls [1]. Fetal hyperinsulinemia may inhibit the normal maturational effect of cortisol on the lung leading to a decreased production of dipalmityl lecithin [38]. While the infant of a non-diabetic mother reaches pulmonary maturity at a mean of 34–35 weeks gestation, the IDM is not considered to be past the risk of pulmonary immaturity until 38.5 weeks gestational age [38]. Additionally, persistent pulmonary hypertension secondary to polycythemia associated vascular sludging may complicate RDS as both polycythemia and RDS are associated with fetal hyperinsulinemia.

Cardiopulmonary adaptation (e.g., transition) in IDMs has been shown to be significantly different than infants born to non-diabetic mothers. The classic findings of interventricular septal hypertrophy caused by glycogen loading of the septum and cardiomyopathy are seen in up to 30% of IDMs [38]. Hypertrophic subaortic stenosis physiology can be observed with severe septal hypertrophy rendering those infants in left ventricular outflow obstructive heart failure. In such a situation, inotropes and hypovolemia worsen the condition, whereas beta-blockers and volume help to alleviate the obstruction. Cardiomyopathy is usually seen in conjunction with septal hypertrophy, although it may appear exclusively [1].

55.5.7 Hyperbilirubinemia

Indirect hyperbilirubinemia is a common finding in IDMs as they have an increased red cell mass, ineffective erythropoiesis, and relative immaturity of hepatic bilirubin conjugation and excretion [1]. A 30% greater source of bilirubin is produced by the increased cell mass, and conjugation and excretion by the relatively immature glucoronosyltransferase enzyme system creates elevated serum unconjugated bilirubin levels. Bilirubin levels should be assessed within the first 24 hours of life and then followed until a peak has been reached. Phototherapy with appropriate hydration monitoring is the mainstay of treatment, but some infants will require exchange transfusion, using the same criteria as in hyperbilirubinemic infants born to mothers without diabetes.

55.5.8 Neuropathology

Central nervous system dysfunction can be seen immediately in the newborn period as well as manifested by neurodevelopmental and behavioral abnormalities not recognized until later infancy or childhood. The IDM is at risk for multiple morbidities that can result in neurologic injury and symptoms. Polycythemia with vascular sludging, glucose and electrolyte abnormalities, birth trauma and perinatal asphyxia due to macrosomia all pose possible etiologies for neurologic symptoms such as seizures, jitteriness, lethargy, tone abnormalities and movement disorders. Different neurologic symptoms typically present at specific time periods based on the etiology. Perinatal depression, birth trauma, and hypoglycemia

all generally present with symptomatology within the first 24 hours of life. Alternatively, symptoms due to hypocalcemia or hypomagnesemia typically present between 24 and 72 hours following birth, as this is the time period at which nadir levels are expected to be reached.

Initial hypotonia followed by increased tone, jitteriness, and seizures are signs and symptoms of brain injury secondary to perinatal asphyxia. The risk for seizures due to birth asphyxia generally peaks 24 hours following birth. Glucose, electrolyte or hematologic abnormalities must be identified and corrected prior to starting anticonvulsant therapy.

Brachial plexus nerve injuries secondary to neck stretching during delivery are more commonly seen in the macrosomic IDM than appropriately grown for gestational age infants. Erb's palsy (roots C5-7), Klumpke's paralysis (roots C-8), diaphragmatic nerve paralysis (roots C3-5), and recurrent laryngeal nerve damage (roots T1-2) have all been reported.

In humans, event-related potential (ERP) studies have indicated that while healthy, term infants born to nondiabetic mothers discriminate their own mother's voice from a stranger's voice, iron deficient IDMs do not show evidence of discrimination, suggesting abnormal cognitive processing [29, 49].

55.6 Long-Term Health Sequelae in IDMs

Periconceptional, fetal, and neonatal events all contribute to the long-term health issues of IDMs. The future risks for development include metabolic syndrome and iron status abnormalities in conjunction with neurodevelopmental problems.

55.6.1 Metabolic Syndrome

In light of the increasing obesity epidemic, concerns have been raised about the long-term health risks of IDMs given their fetal exposure to an abnormal metabolic milieu and macrosomic neonatal body habitus. Several studies have focused on the initial growth and ultimate likelihood of IDMs to go on to develop metabolic syndrome, characterized by increased insulin resistance often seen in the setting of obesity, hypertension, and dyslipidemia [50–54]. A longitudinal study focusing on growth of Pima Indian children showed significantly different patterns of growth of offspring of diabetic mothers compared to offspring of nondiabetic mothers. Offspring of diabetic mothers showed significant "catch-down" growth during their first 1 ½ years of life, but by age seven had greatly exceeded the weight of offspring of nondiabetic mothers [50]. Another longitudinal study identified a trend toward a higher incidence of insulin resistance (defined as a fasting glucose/insulin ratio of <7) in eleven-year-old children who were large for gestational age at birth compared to those who were appropriate despite no difference in obesity. The authors also demonstrated by multivariate logistic regression that childhood obesity and the combination of large for gestational age status and maternal gestational diabetes was associated with insulin resistance [51]. Finally, a review of studies examining the consequent development of metabolic syndrome in gestationally diabetic mothers and their offspring showed that metabolic syndrome in children with increasing age is related to maternal gestational diabetes, maternal glycemia in the third trimester, maternal obesity, neonatal macrosomia, and childhood obesity [52].

55.6.2 Neurodevelopment

Because of the many morbidities for which IDMs are at increased risk, the long-term motor and cognitive developmental trajectories of these children have been studied in comparison to infants born to nondiabetic mothers. Acute perinatal events as well as abnormal intrauterine environmental affects can contribute to the long-term risks to brain development. Both newborn studies and longitudinal research have demonstrated significant neurodevelopmental differences between these groups of offspring.

For infants that experience seizures in the newborn period, the etiology of the seizures is critical for determining prognosis. Seizures caused by metabolic abnormalities such as hypoglycemia or hypocalcemia have a 10% to 50% risk of later developmental delays. In contrast, infants who have seizures due to hypoxic-ischemic encephalopathy carry an 80% risk for developmental problems [55]. Transient hypoglycemia in the presence of large-for-gestational-age infants born to nondiabetic mothers has been shown to have no harmful impact on psychomotor development at four years [56]. Symptomatic, severe hypoglycemia (blood glucose <35 mg/dL), however, has been shown to be associated with neurodevelopmental abnormalities and white matter injury identified on MRI [57]. MRI is recommended for routine evaluation of a newborn infant with symptomatic hypoglycemia to elucidate any cerebral injury [58].

Specific areas of neurodevelopment of offspring born to diabetic mothers have been shown to correlate with the degree of maternal glycemic control [59–62]. Problems with attention span and motor functions appear to be present even in well-controlled mothers. Gross cognitive ability has appeared to be maintained unless pregnancies are associated with significant maternal nephropathy or hypertension or with neonatal iron deficiency [62] in which case the effects negatively correlate with the level of maternal glycemic control [59, 62]. Another longitudinal study that followed IDMs until 11 years of age showed that poorly controlled antepartum maternal glucose and lipid metabolism correlated with poorer child performance on standard measures of neuropsychological functioning [60].

55.7 Summary

Advances in medical care of pregnant women with diabetes have lead to improved outcomes for mothers and their offspring. Despite this trend, the rising obesity epidemic has the potential to alter the course of progress. Metabolic Syndrome characterized by insulin resistance and type II diabetes mellitus will likely continue to rise over the ensuing decade making glycemic control a more universal issue. Health care providers who care for pregnant women ought to stress the possible implications of poor glucose control, including fetal hypoxia and iron deficiency that can affect long-term neurodevelopmental trajectories of their infants. Women who first present with insulin resistance in pregnancy, particularly those who are overweight or obese, need to be followed for the possibility of developing type II diabetes later in life. Additionally, health care providers of infants and children ought to be aware of the possible sequelae for offspring of diabetic mothers both in the neonatal period as well as long-term. Atypical neurodevelopment and the propensity for a metabolic derangement are two arenas that warrant further research and attention as these infants are followed throughout childhood.

This work was sponsored in part by grants to MKG from the National Institutes of Health (HD-29421-14; NS-34458-14; HD-54490; and RR-00400-39).

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56

Lung Development and Pulmonary Malformations

Corrado Moretti and Paola Papoff

56.1 Fetal Stage of Lung Development

56.1.1 Introduction

In the early weeks of gestation the human lung originates as a ventral endodermal pouch from the primitive foregut; it thereafter continues to grow through to adulthood until it reaches an exchange surface area of around 70–100 m^2; the air-blood barrier is some 0.2 microns thick, 1/50 of the thickness of a sheet of tissue paper.

From the structural standpoint the lung is made up of one part that serves to convey gas extending from the trachea to the terminal bronchioli, characterized by a series of 23 branchings of the respiratory tree, and of an alveolar zone where gas is exchanged, where the acinus is the functional unit. The acinus comprises the respiratory bronchioles, often with alveoli on the walls, the alveolar ducts and the alveoli themselves, which are characterized by a hexagonal structure (Fig. 56.1). The surface area of this structure is vast and maintains a constant relationship with body mass during postnatal growth (about 1 m^2/kg).

The development of the human lung begins when the lung bud appears in the embryo and ends in early childhood after relatively stable and progressive growth. Even though birth is an event that implies dramatic changes in the functions of this organ, it should not be considered as a precise point of transition from one stage of development to another. Nevertheless, since this is an important event, the artifact of dividing lung development into a prenatal and postnatal stage is justified, also to emphasize that at the end of gestation, this organ has not yet reached maturity. Indeed, at the time of writing we cannot define exactly when the lung reaches maturity [2]. Prenatal development of the respiratory system is in turn conventionally divided into five periods [3]; actually, a precise watershed between these periods does not exist, but their identification can however simplify the understanding of the morphogenesis of the lung.

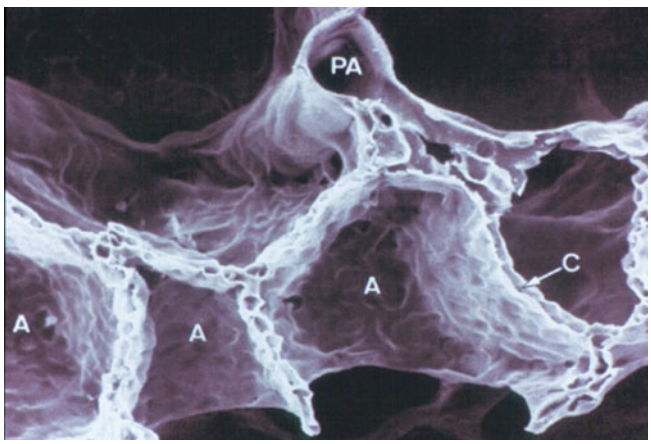

Fig. 56.1 The electron micrograph shows some alveoli (**A**) with a dense capillary network (**C**) between their walls. The capillaries stem from a branch of the pulmonary artery (**PA**). Reproduced from [1], with permission

56.1.2 Embryonic Phase (3rd–7th week)

The lung bud appears on the 26th day of gestation and is formed from relatively undifferentiated epithelial cells that expand into the surrounding embryonic connective tissue or mesenchyme (Fig. 56.2a,b). The bud begins to grow in length and to branch until, towards the 5th week, it forms five small saccular structures, two on the left and three on the right, which are the beginnings of the future secondary bronchi and

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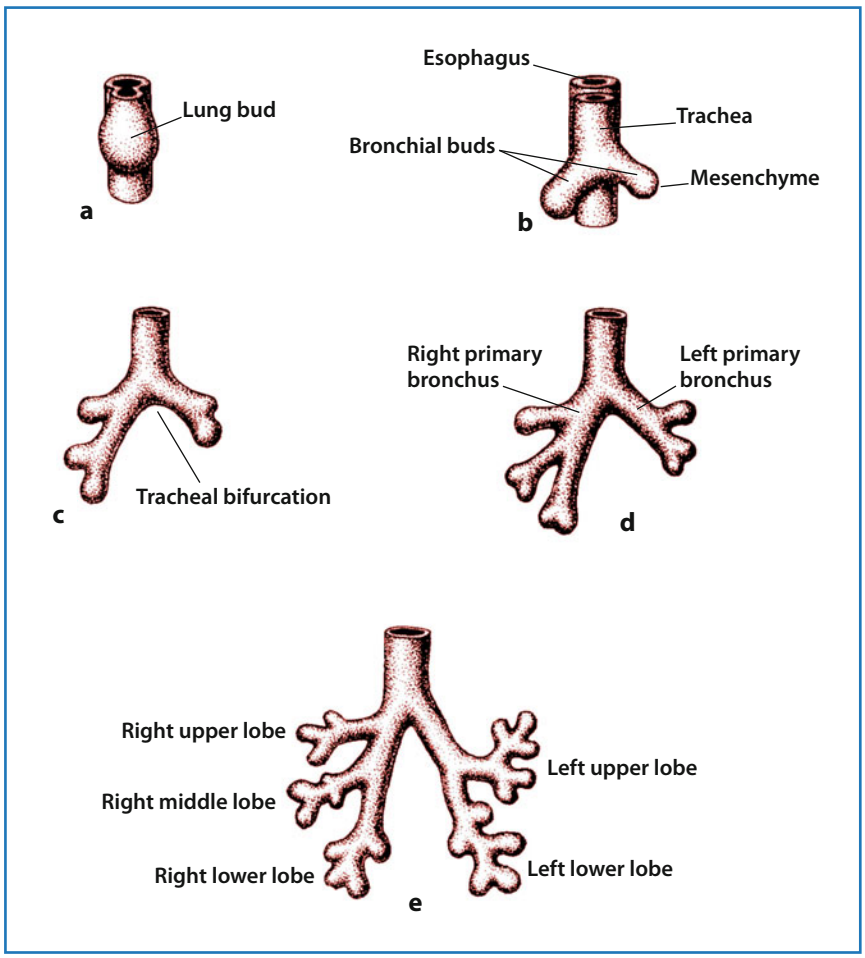

Fig. 56.2 Successive stages in the development of the bronchi and future lobes of the lungs: **a** and **b**, four weeks; **c** and **d**, five weeks; **e**, six weeks. Modified from [4]

of the future lobes of the mature lung (Fig. 56.2c,d). The developing airways will undergo further branching and are accompanied by the pulmonary arteries that derive from the sixth aortic arches.

Interactions between the epithelial cells of endodermic origin that form the respiratory tree and the mesenchymal tissue of mesodermal origin covering it are of basic importance for the branching of the airways [5]; this process is often referred to as "cross-talk". The process is controlled through a multitude of molecular factors that include transcriptional regulators, growth factors, morphogens and extracellular matrix molecules, all of which must be carefully controlled in both space and time to form a properly functioning lung. If some of these developmental factors do not function at the right time and/or place, then lung defects may occur.

Towards the end of the 6th week of gestation, the main airways are complete, including the segmental and sub-segmental bronchi (Fig. 56.2e).

Developmental anomalies occurring in these first weeks of gestation may lead to many congenital malformations such as pulmonary agenesia, laryngeal or tracheal stenosis or athresia, tracheo-esophageal fistula, tracheo-malacia or broncho-malacia, bronchial malformations and ectopic lobes (Table 56.1).

Table 56.1 Stage of development of congenital pulmonary malformations

Embryonic
Pulmonary agenesis
Tracheal or laryngeal agenesis or stenosis
Tracheo- or bronchomalacia
Bronchial malformations
Ectopic lobes
Pseudoglandular
Cystic adenomatoid malformation
Pulmonary sequestration
Lung cysts
Congenital pulmonary lymphangiectasia
Congenital diaphragmatic hernia
Canalicular
Pulmonary hypoplasia
Saccular/Alveolar
Pulmonary hypoplasia
Alveolar capillary displasia

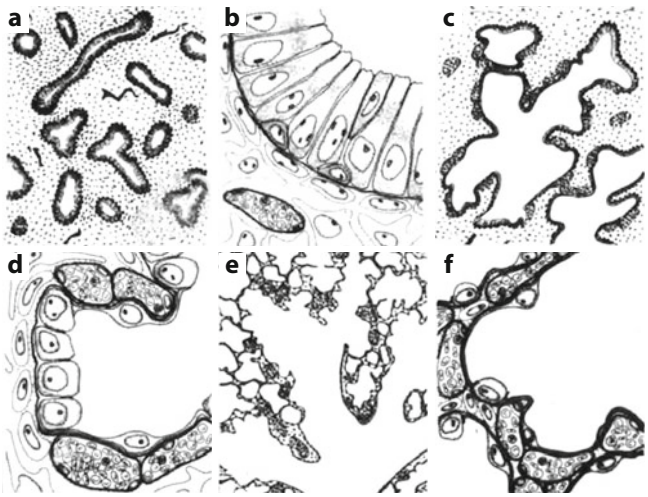

Fig. 56.3 Drawing that illustrates the light microscopic appearance of human lung during the glandular (**a**, **b**), canalicular (**c**, **d**) and saccular (**e**, **f**) stages of in utero development. Undifferentiated columnar cells of the early bronchi (**b**) rest upon a basal lamina, which has been emphasized. Mesenchymal cells and capillaries are present below the basal lamina. **d** shows that the epithelium overlying proliferating blood vessels thins to a squamous Type I morphology and that the most distal cells retain a cuboidal shape. At the saccular state (**f**), the septa are very thinned and the air spaces are lined by differentiated Type I and Type II cells. The low and high magnification drawings are shown at approximately the same magnification in each panel. Modified from [6]

56.1.3 Pseudoglandular Phase (6th-17th week)

During the pseudoglandular period the airways continue to divide until they form terminal bronchioles; by the end of this period the bronchial branching process is complete and the airways will continue to grow in proportion with the increase in pulmonary volume.

Since the newly formed epithelial tubules are surrounded by a considerable amount of mesenchyme, they make the lung look like a gland, hence the name of this phase (Fig. 56.3a,b). The proximal airways are covered by an epithelium that gradually becomes thinner towards the periphery, where the cells take on a cuboidal shape.

During this period the blood vessels develop in parallel to the airways so that, by the end of this phase, their hierarchical structure is already comparable to that of the adult [7]. Also, the development of the nerves in these structures is already fairly complete.

Alterations in the development of the lung during the pseudoglandular period may give rise to many congenital malformations such as pulmonary sequestration, cystic adenomatoid malformation, pulmonary cysts and congenital pulmonary lymphangectasia (Table 56.1). In the early stages of this period, the pleuro-peritoneal cavity starts dividing into the peritoneal and the pleural cavities due to the development of the diaphragm; when it does not divide correctly, the abdominal content in the chest becomes herniated (diaphragmatic hernia).

56.1.4 Canalicular Phase (16th-26th week)

The canalicular period is characterized by the appearance of vascular canals (capillaries), hence the name, and by further development of the distal airways where the primitive acinar structures appear, each made up of a respiratory bronchiole, an alveolar duct and rudimentary alveoli or saccules [8]. The development of the terminal airways and of the capillary network determines both the progressive thinning of the interlying mesenchyme and the formation of the alveolar-capillary membrane (Fig. 56.3c,d). This phase is also characterized by the differentiation from cuboidal type-II to squamous type-I cells in the distal epithelium. Differentiation of these cell types is an important step as the type-II cells will be required to produce and secrete surfactant and the type-I cells will form the thin cell layer to support future gas exchange.

In this period the presence of masses inside the chest and/or changes in the physical forces, essential for lung development, produced by the presence of the pulmonary liquid and by the breathing movements of the fetus, may interfere with the development of the respiratory system and give rise to pulmonary hypoplasia.

56.1.5 Saccular Phase (25th–38th week)

This phase is characterized by marked dilation of the terminal airways and resultant formation of saccules [3], with a considerable increase in lung volume and in the surface area for gas exchange together with a further thinning of the alveolar-capillary membrane (Fig. 56.3e,f). In this way also the microcirculation structure is modified because the capillary networks surrounding the saccules gradually come closer to each other and, at the end of the process, the interstitial septa, or primary septa, delimit a double network of capillaries necessary for the subsequent formation of the secondary septa [2].The formation of the final number of alveoli, around 200–300 million, happens during the alveolar phase, which begins towards the end of gestation and ends during early infancy. The alveoli are formed through the subdivision of the saccules into subunits, thus considerably increasing the exchange surface area. It must be pointed out that, given the great difficulty in carrying out histological measurements and in standardizing findings, there is still controversy over the beginning and the end of the alveolar phase and over the final number of alveoli.

The formation of alveoli is to be considered as mainly a postnatal event since most form during the first 24–36 months after birth with a subsequent slow increase in number up to around the 8th year of life [9].

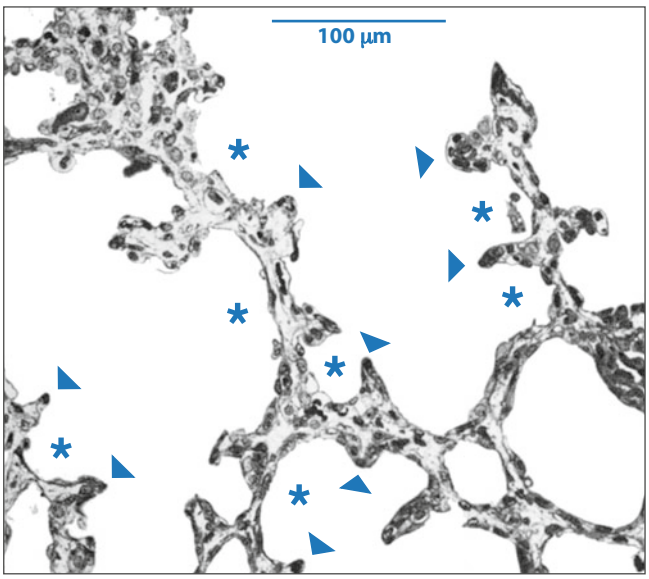

Fig. 56.4 Light micrograph of human lung. Air spaces are subdivided into small alveoli (*asterisks*) by secondary septa (*arrowheads*) projecting from the primary one

56.2 Postnatal Lung Development

56.2.1 Alveolar Phase and Development of the Pulmonary Vasculature (36th week of Gestation–24 months after birth)

Crests, or secondary septa, grow out from the intersaccular walls, or primary septa (Fig. 56.4), appearing as small ridges in epithelium [10]. Electron micrograph imaging shows the secondary septa to be formed by a thin layer of connective tissue flanked by capillaries on both sides and characterized by the presence of elastin at the tip. Elastin appears to have the function of supporting the outgrowth of the epithelium, and its presence is an essential prerequisite for the formation of the secondary septa, which takes place only after its deposition. Also, the capillary network gradually develops until maturity: in the first few weeks after birth, the interstitial layer of the septa gradually thins, drawing the two capillary networks closer until they finally merge. This transformation from a double to a single layer of the capillary network contained inside the septa is the last stage of pulmonary development [11].

56.3 Physical Determinants of Lung Development

Physical forces exerted on the lung during gestation are crucial to normal development. There are two main physical factors that aid lung development: lung fluid and fetal breathing movements (FBM).

During fetal development the lung is a secretory organ that exhibits breathing-like movements without contributing to respiratory gas exchange. The lung fluid is produced by epithelial cells, especially those in the distal airways, through chloride secretion, and it flows to the upper airway where it is either swallowed or released into the amniotic space. The fluid is rich in chloride and low in bicarbonate and proteins. The rate of fluid production increases in fetal lambs from approximately 5 mL/kg at mid-gestation to more than 20 mL/kg near term, with hourly rates increasing from 2 mL/kg/h to 5 mL/kg/h, respectively [12]. This increase mirrors the progressive expansion of the contact surface between alveolar epithelium and microcirculation, due to the proliferation of pulmonary capillaries and to the growth of the terminal saccules.

The production of pulmonary fluid is essential for the normal development of the respiratory apparatus since it determines the form and volume of its peripheral units; experimental evidence shows that extended drainage of tracheal fluid causes pulmonary hypoplasia. The amount of pulmonary fluid that is present inside the respiratory tree depends on the balance between production at the level of the alveolar epithelium and resistance to its outflow exerted by the upper airway [13]; its volume at the end of gestation is around 20–30 mL/kg, a value that is comparable to that of the functional residual capacity (FRC) of a normal lung.

The other physical factor that is critical for normal lung development is FBM, periodic rhythmic contractions of the diaphragm that maintain adequate fluid volume and lung distension. During pregnancy, the fetus spends most of its time, around 30–40% during the third quarter, performing breathing movements even though ventilation is exclusively a placental function [14]. Fetal breathing appears to be related to the behavioral state of the fetus. As the fetus approaches term, the incidence of these movements is considerably greater during active periods than during periods of quiet activity [15].

Observations of human fetuses with congenital anomalies and of experimental animals show that the breathing movements of the fetus produce distension forces that stimulate cell multiplication [16] and are necessary to ensure an adequate development of the muscular and respiratory apparatus for postnatal life. Pulmonary hypoplasia is a disorder that is frequently associated with oligohydramnios and experimentally it can be determined in the uterus through bilateral phrenectomy. These observations seem to confirm the importance of fetal respiratory activity for the development of the lung, which could undergo alterations when this activity is prevented or when there are obstacles [17]. The physiological fluctuations of PaO_2 and of $PaCO_2$ do not influence the respiratory activity of the fetus, which stops if the PaO_2 drops to a level of 16–18 mmHg. Hypoxia therefore has an inhibiting effect on breathing movements, which is not to be interpreted as a sign of neuronal immaturity but rather as a response aimed at reducing the consumption of O_2 by the fetus. In fact, intrauterine respiratory

activity demands up to 15–30% of the total O_2 available. By contrast, the fetus responds to an increase in $PaCO_2$ with a corresponding increase in respiratory activity and the opposite occurs in the case of hypocapnia. These observations suggest that the central chemoreceptors are active in fetal life. Maternal ingestion of alcohol or sedatives has been shown to decrease breathing movements, but drugs such as caffeine increase them. Maternal smoking also decreases fetal breathing movements through several mechanisms including decreased uterine blood flow and hypoxia [18].

The pressure inside the fetal respiratory system varies depending on the presence or absence of breathing movements. During the periods in which the fetus does not practice breathing, pressure remains constant [19]: if the amniotic pressure is considered to be zero, the pressure inside the trachea is around 1–2 mmHg greater, while the intrapleural pressure is about 0.7 mmHg lower. The positive pressure inside the respiratory tree is modulated mainly by the degree of resistance of the larynx. Similarly, the negative endopleural pressure is the product of the balance between elastic return of the lungs and of the thoracic cage. The resulting force between endotracheal positive pressure and endopleural negative pressure is a transpulmonary positive pressure of around 2.5 mmHg, a force that keeps the lungs expanded hence stimulating their growth. During the periods when the fetus performs FBM, the glottis dilates and resistance decreases at the larynx, but the rhythmic contractions of the diaphragm attenuate the outflow of fluid from the trachea and restrict the reduction of pulmonary volume [20].

56.4 Pulmonary Malformations

Knowledge of the various phases of pulmonary development allows us to understand the pathophysiology of the various congenital malformations of this organ, many of which, thanks to the considerable development of prenatal ultrasonography, may be diagnosed as early as between the 18th and 20th weeks of gestation.

56.4.1 Intrapulmonary Cystic Malformations

Intrapulmonary cystic malformations comprise a spectrum of congenital malformations of the lower respiratory tract that have in common the fact that cysts may represent (but not always) a prominent part of the anomaly; these include congenital cystic adenomatoid malformation (CCAM), bronchopulmonary sequestration (BPS), congenital lobar emphysemas (CLE), and bronchogenic cysts.

Intrapulmonary cystic malformations have been increasingly detected at midgestation due to improvements in ultrasound technology and the routine use of ultrasound as a screening test. The different postnatal diagnostic entities are difficult to differentiate prenatally and there is confusion in terms of how these malformations should be described; recently a new nomenclature using the terms "congenital thoracic malformation" (CTM) and "congenital large hyperlucent lobe" have been proposed [21]. It has been suggested that many, if not all of these defects, may represent a continuum of anomalies of fetal lung development, despite some diversity in morphology. Fetal airway obstruction probably plays a prominent role in their pathogenesis [22], but the mechanism by which bronchial athresia might lead to different fetal chest masses remains unknown.

The widespread use of antenatal ultrasound with Doppler studies has led to a better understanding of the development of some of these anomalies and to an improvement in their management. Antenatal fast magnetic resonance imaging is also an important adjunct to prenatal ultrasound in the evaluation of CTM: it may provide further information on the nature of the lung anomaly, it may help to differentiate lung lesions from extrathoracic abnormalities such as congenital diaphragmatic hernia, and it may be useful in planning pre- or postnatal surgery.

Large congenital malformations may compress the ipsilateral lung or even the contralateral lung via mediastinal shift thus causing lung hypoplasia. Compression of the esophagus may result in polyhydramnios; the resulting uterine distension may induce premature labor. Impairment of cardiac return due to the vena cava and to cardiac compression is responsible for fetal hydrops with ascites, pleural and pericardial effusions, and skin and scalp edema.

CTM may change in size and/or appearance during pregnancy, but the growth pattern is rather unpredictable. There is a wide spectrum of clinical severity, with a relationship between size of the malformation, concomitant lung hypoplasia and the occurrence of pulmonary hypertension in the newborn period. Most fetuses with CTM have a good outcome, and an initially large lesion does not necessarily correlate with a poor prognosis [23, 24]. In fact, many lesions (both CAM and BPS) decrease in size over time [23, 25–28], and some even disappear completely on serial prenatal ultrasound scans toward the end of pregnancy [23, 25, 29–31]. However, although these lesions are often undetectable on postnatal ultrasound and chest X-ray examination, in the vast majority of cases they can be detected by computed tomography (CT) scans or magnetic resonance imaging [26, 32–35]. Thus all patients with prenatally detected CTM require postnatal evaluation, and documentation of true resolution must be confirmed, preferably by CT scans. It is also important in the postnatal period to seek an anomalous blood supply in patients who have CTM, even if prenatally color flow Doppler studies fail to identify a systemic arterial blood supply.

Therefore, most fetuses with a CTM will do well during pregnancy. However, if hydrops develops, the risk of fetal or neonatal death is extremely high [23, 25, 36]: most current reports suggest that a large cystic mass volume (specifically when measured as a ratio of normal lung to thorax) and

hydrops are the only indications for fetal intervention [27, 37]. In intrathoracic lesions with cystic parts, prenatal needle aspiration of the fluid has been performed but often leads to rapid re-accumulation. Therefore, fetal surgery with the implantation of shunts between the cyst and the amniotic space has successfully reduced the size of the lesion and improved hydrops fetalis; however, this intervention may cause fetal death [30, 38].

After 32 weeks of gestation, early delivery should be considered in hydropic fetuses and, furthermore, when severe RDS is expected, the ex-utero intrapartum therapy (EXIT) procedure may be considered to allow interventions such as drainage of large pleural effusions or even resection of an affected lobe [39]. A recent paper has reported 100% survival in children with large intrathoracic cystic masses causing hydrops fetalis after maternal betamethasone therapy [40]. Although the effect of steroid treatment on CCAM growth was variable, resolution of hydrops was seen in 80% of steroid-treated patients.

The postnatal clinical appearance of CTMs may vary from immediate respiratory distress at birth to an incidental finding on a chest radiograph at any age. In the neonatal period a small number of patients with large lesions require surgery, which is the accepted standard of care for all symptomatic lesions. Many children, however, will be asymptomatic at birth and in the neonatal period, and there is controversy about the management of these newborns: most authors would recommend resection of CAMs, cysts and BPSs if the lesions can be detected postnatally, because of complications such as infections, hemorrhage, pneumothorax, sudden respiratory failure, and malignant transformation [41–43]. Moreover, conservative management of these lesions with repeated CT scans causes significant radiation exposure. Successful expectant long-term management has been reported particularly for late presenting asymptomatic or oligosymptomatic lobar emphysema [44].

CAMs, BPSs and cystic malformations can be removed surgically before 6 months of age [27, 45] with excellent results, either by open thoracotomy or, more recently, by using the minimally invasive video-assisted thoracoscopic approach [46, 47].

56.4.2 Congenital Cystic Adenomatoid Malformation

CCAM is characterized by an adenomatoid proliferation of terminal bronchioles with the formation of solid, cystic or mixed masses inside the pulmonary parenchyma without subsequent alveolar differentiation [48]. This lesion is usually unilateral, without a right or left prevalence, and it affects a single lobe; vascularization originates from the bronchial circulation, but in rare cases it may depend on an anomalous systemic vessel.

56.4.2.1 Etiology and Pathogenesis

The pathogenesis of CCAM is still unclear, but it is likely to be due to a failure of normal interaction between the mesenchymal tissue and epithelial cells during early fetal life.

56.4.2.2 Clinical Aspects

The congenital cystic adenomatoid malformation was originally classified by Stocker into three distinct histological subtypes, and recently expanded to five [49]:

- type 0: involvement of all lung lobes, incompatible with life (<2%);
- type 1: single or multiple cysts, size >2 cm, lined by pseudostratified columnar epithelium; wall of fibromuscular and cartilaginous tissue (60–70%);
- type 2: single or multiple cysts (<2 cm) lined by cuboidal or columnar epithelium (15–20%);
- type 3: predominantly solid lesions, with small (<0.5 cm) cysts, lined by cuboidal epithelium (5–10%). On the whole, this constitutes a solid mass and is the form that has the worst prognosis;
- type 4: large air-filled cysts, lined by flattened epithelial cells (<10%).

This classification can obviously only be applied to resected lung specimens and it is inappropriate for describing fetal lung lesions. The emerging consensus is that imaging findings should simply describe without attempting to make a pathological diagnosis, as done by Adzick in his classification [50] in which he simply differentiated prenatally detected cystic lesions into two types:

- macrocystic (single or multiple echogenic cysts >5mm)
- microcystic (smaller echogenic cysts <5 mm).

56.4.2.3 Differential Diagnosis

The chest radiograph and clinical symptoms of CCAM may be easily confused with those of a diaphragmatic hernia: the insertion of a nasogastric tube and instilling a few mL of barium may help locate the stomach and hence facilitate differential diagnosis (Fig. 56.5). Ultrasound and CT scans are also useful in determining the size of normal lung and the position of the diaphragm.

56.4.2.4 Therapy and Treatments

The risk of infections, associated with the risk of malignant degeneration of the adenomatoid tissue into rhabdomyosarcoma or into bronchioloalveolar carcinoma or into other mesenchymal malignancies, a complication described both in children and in adults [41, 43], is to be considered when deciding on surgery in an asymptomatic patient. Also, in asymptomatic cases, a chest CT scan within one month of birth is mandatory, even if resolution is noted on prenatal scanning

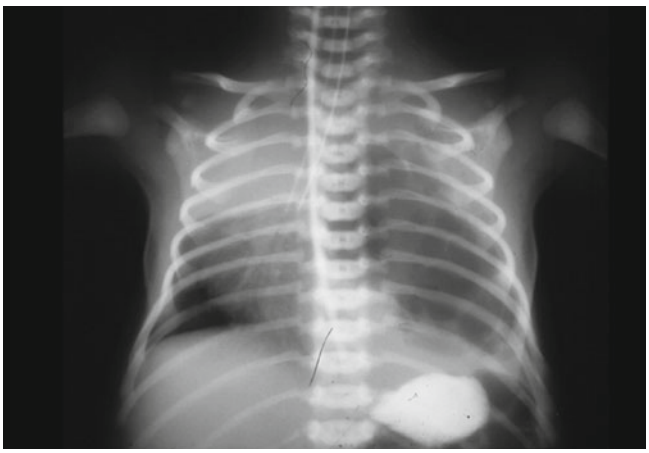

Fig. 56.5 Left lower lobe CCAM with mediastinal shift to the contralateral side

[41] and bearing in mind that a true resolution of these lesions is exceptional.

When needed, assisted ventilation in these patients may determine a progressive distension of the cysts with a life-threatening mediastinal shift: one-lung ventilation may be carried out in order to achieve perioperative stabilization (Fig. 56.5). High-frequency oscillatory ventilation may also be successfully used in patients with pulmonary hypertension [51].

56.4.3 Bronchopulmonary Sequestration

The term bronchopulmonary sequestration (BPS) was introduced by Pryce in 1946, who took the term from the Latin word sequestrate which means "to separate". He used it to define a portion of the pulmonary tissue that does not work and is not linked to the bronchial tree.

56.4.3.1 Etiology and Pathogenesis

The pathogenesis may be referred to an anomaly in the development of the primitive lung bud with the formation of an ancillary structure that has preserved the original arterial supply and that grows inside the pleural cavity in close contact with the normal lung (intralobular sequestration), or it may remain separate and be wrapped in its own visceral pleura (extralobular sequestration).

56.4.3.2 Clinical Aspects

Intralobular sequestration is the form most frequently found and is usually located in the lower lobes; extralobular sequestration is located almost constantly on the right, between the diaphragm and the lower lobe, and is often associated with other congenital malformations, in particular diaphragmatic hernia. This malformation has its own vascular supply that usually originates from the lower chest or upper abdominal aorta or one of its major branches, rather than from the pulmonary artery. Usually the venous blood is normally drained into the right atrium, but occasionally the venous blood may also drain abnormally into the left atrium, the vena cava (the so called Scimitar syndrome for its characteristic chest radiograph appearance), or into the azygos system [52]. The size of abnormal arteries and veins and consequently the blood flow through the malformation is considerable and may cause heart failure and massive arteriovenous shunting [53]. Recently many papers have described cystic lung lesions, defined "hybrid lesions", with coexisting features of both CCAM (the histological features are mostly Stocker type 2 lesions) and BPS, suggesting that they may represent the two ends of a broad spectrum of disorders [27, 51].

Only a small percentage of children with intralobular sequestration have a symptomatology characterized by respiratory distress in the neonatal period. In most cases the malformation is asymptomatic for a long time and then it appears later in life with localized recurrent pneumonias, fever and occasionally hemoptysis. By contrast, extralobular sequestration rarely presents respiratory symptoms and may also be found incidentally on routine chest radiography. BPSs have rarely been linked with malignancy and most likely these tumors develop in hybrid lesions [54].

56.4.3.3 Differential Diagnosis

The initial detection of BPSs is currently obtained by intrauterine sonography (Fig. 56.6) and Doppler ultrasound is useful as it shows the characteristic vascular abnormality. Postnatally, intralobular sequestration should be suspected when the chest radiograph shows a dense lesion on the posteromedial part of the left or, less frequently, right lower zone of the lung. Multi-detector CT evaluation with intravenous

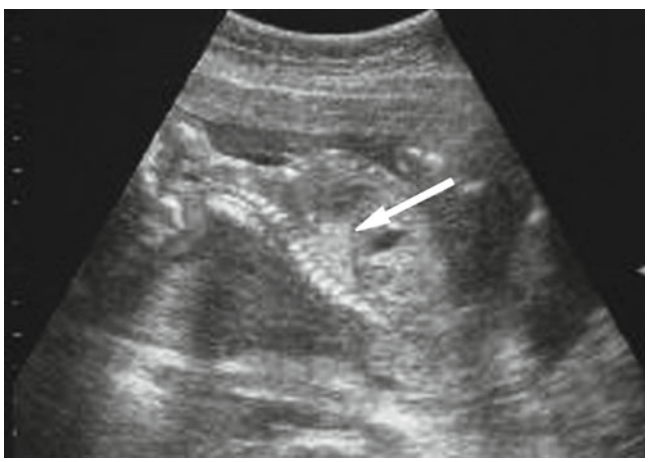

Fig. 56.6 Pulmonary sequestration: midtrimester fetal sonogram shows a triangular echogenic mass astride the left diaphragm

contrast for CT angiogram is useful in establishing the diagnosis: this study provides the best evaluation of the vascular anatomy of the lesion, of the appearance of the lesion itself, of the diaphragm and of the remainder of the lung [55].

56.4.3.4 Therapy and Treatments

Surgery is recommended once the infant becomes symptomatic and should be undertaken only after assessing the characteristics of the arterial supply and of the venous drainage.

56.4.4 Congenital Lung Cysts

Congenital lung cysts are divided into bronchogenic, alveolar and combined forms. They may lie outside the normal lung structure (extrapulmonary), or within it (intrapulmonary). The cysts tend to be limited to one lung and are not associated with cystic disease elsewhere in the body. Bronchogenic cysts may vary notably in size and are rare in the newborn period.

56.4.4.1 Etiology and Pathogenesis

Congenital lung cysts result from abnormal budding of the foregut; the position within the branching pattern indicates when the cyst developed.

56.4.4.2 Clinical Aspects

Such lesions are found in the mediastinum in over 2/3 of cases. They are usually located in the right para-tracheal or carinal regions, but can also be found within the parenchyma of the lung. The cysts contain a lining of respiratory epithelium and have cartilage, smooth muscle and glands in the wall [48]. They are usually unilocular, filled with fluid or mucus and generally do not communicate with airways. Location of the cyst is important in determining the clinical presentation: a bronchogenic cyst may cause airway compression resulting in cough, wheeze, dyspnoea, or even respiratory distress and those located in the region of the carina may determine lobar emphysema. Secondary infection of the cyst is also a very frequent complication and may cause acute distension with exacerbation of symptoms. In addition, peptic ulceration may develop in cysts containing gastric mucosa [56].

56.4.4.3 Differential Diagnosis

Radiographic findings are variable and range from a rounded mass to atelectasis or hyperinflation of a lobe or an entire lung (Fig. 56.7). The diagnosis is usually established from the chest X-ray, but the lesion must be differentiated from congenital lobar emphysema (CLE), acquired cysts complicating pulmonary interstitial emphysema (PIE) and bronchopulmonary dysplasia (BPD). Usually the latter two conditions can easily be differentiated through patient history.

56.4.4.4 Therapy and Treatments

All congenital lung cysts require surgical resection because they do not spontaneously regress, and even those that are asymptomatic may cause problems as a result of compression or infection [43].

56.4.5 Congenital Lobar Emphysema

Congenital lobar emphysema (CLE), defined as a postnatal overdistension of one or more lobes of a histologically normal lung, is a rare cause of respiratory failure in the newborn, but in most patients the diagnosis is made before six months of life [57].

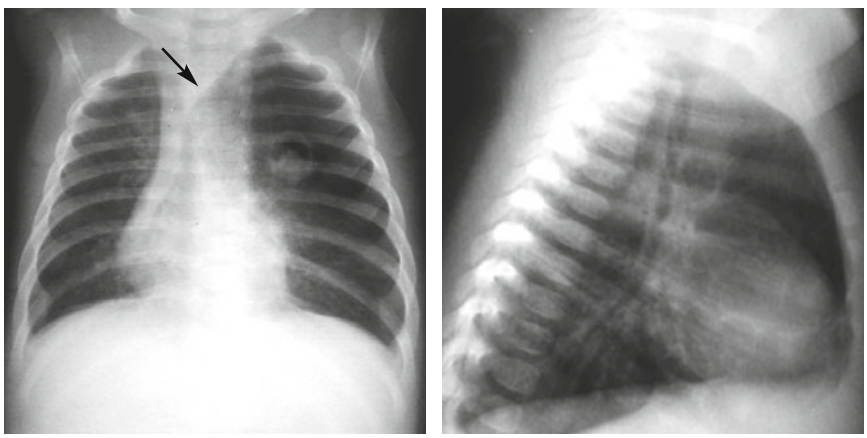

Fig. 56.7 Left bronchogenic cyst. The *arrow* shows upper lobe emphysema

56.4.5.1 Etiology and Pathogenesis

CLE may result from a malformation in the bronchial cartilage, with no or incomplete rings. It also may result from extrinsic bronchial obstruction caused by anomalous vessels or from intrathoracic masses such as bronchogenic cysts. The etiology of the intrinsic bronchial obstruction is thought to be a localized interruption of the blood supply to an already formed bronchus, resulting in bronchial infarction and luminal obstruction or developmental abnormalities of the bronchial cartilage after completion of bronchial branching [58].

56.4.5.2 Clinical Aspects

The left upper lobe is most often involved (Fig. 56.8), followed by the right middle lobe; involvement of the lower lobes is very rare. Dyspnea, tachypnea, wheezing, tachycardia and cyanosis are among the most common presenting symptoms. Chest radiograph, computed tomography (CT), ventilation scintigraphy and bronchoscopy are usually sufficient for diagnosis. The chest radiograph will demonstrate marked hyperinflation of the affected lobe with the adjacent lobe collapsing down or up and mediastinal shift to the controlateral side.

56.4.5.3 Differential Diagnosis

CLE can easily be confused with pneumothorax, but in the latter there are no bronchovascular markings in the radiolucent area [43]. A CT scan is useful in the differential diagnosis of a mediastinal mass.

56.4.5.4 Therapy and Treatments

The traditional treatment for most cases of CLE with respiratory distress is lobectomy. Conservative treatment is possible in asymptomatic or mildly symptomatic cases [59].

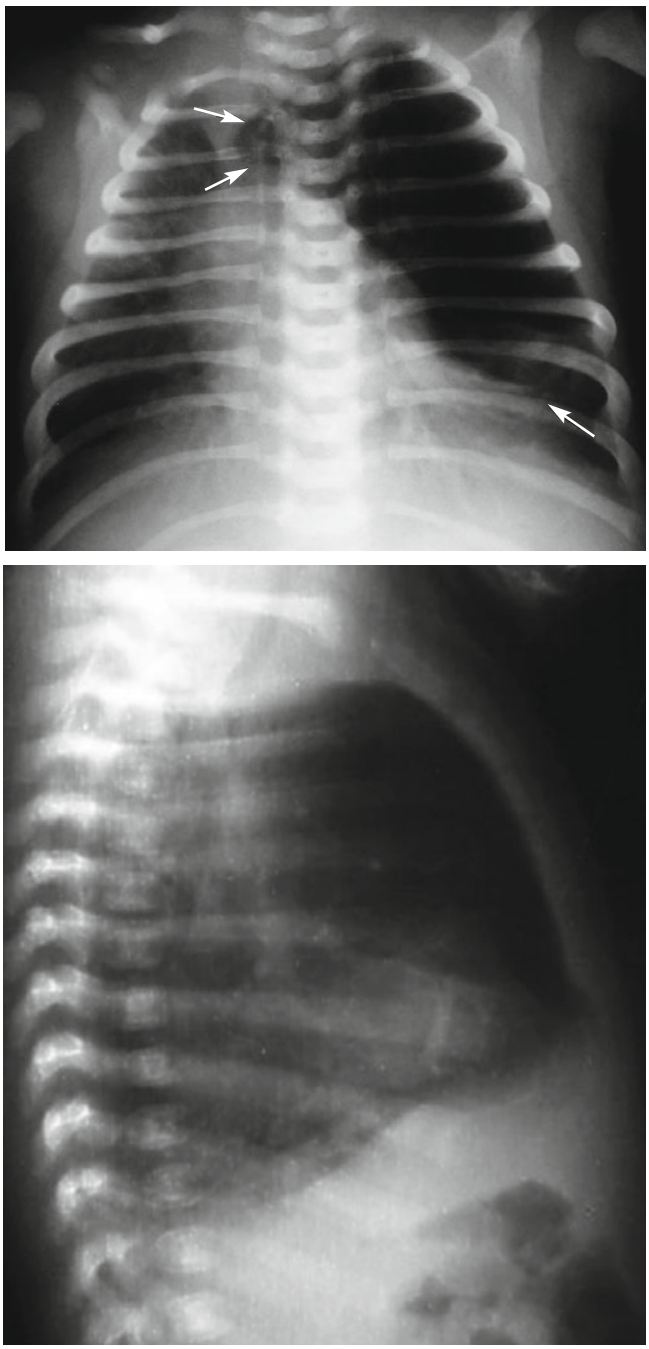

Fig. 56.8 Congenital left upper lobar emphysema. The *upper arrows* show retrosternal hernia. The *lower arrow* shows the collapse of the lower lobe

56.4.6 Pulmonary Agenesis

Pulmonary agenesis may be classified morphologically by the extent to which bronchopulmonary tissue is absent [48]. Pulmonary agenesis is divided into (1) bilateral complete agenesis, (2) unilateral agenesis with (a) complete absence of lung and bronchus and no vascular supply to the affected side (agenesis), (b) rudimentary bronchus with complete absence of pulmonary parenchyma (aplasia), (c) presence of variable amounts of bronchial tree, pulmonary parenchyma and supporting vasculature (hypoplasia), and (3) lobar agenesis. The incidence may range between 0.0034 and 0.0097% [60]. The left lung is slightly more affected than the right. Isolated agenesis of a lobe or lobes is more common than total unilateral lung agenesis. This abnormality occurs more often on the right than on the left, involving the upper and middle lobes.

56.4.6.1 Clinical Aspects

Unilateral pulmonary agenesis may present with severe respiratory distress at birth or may be asymptomatic. In this latter

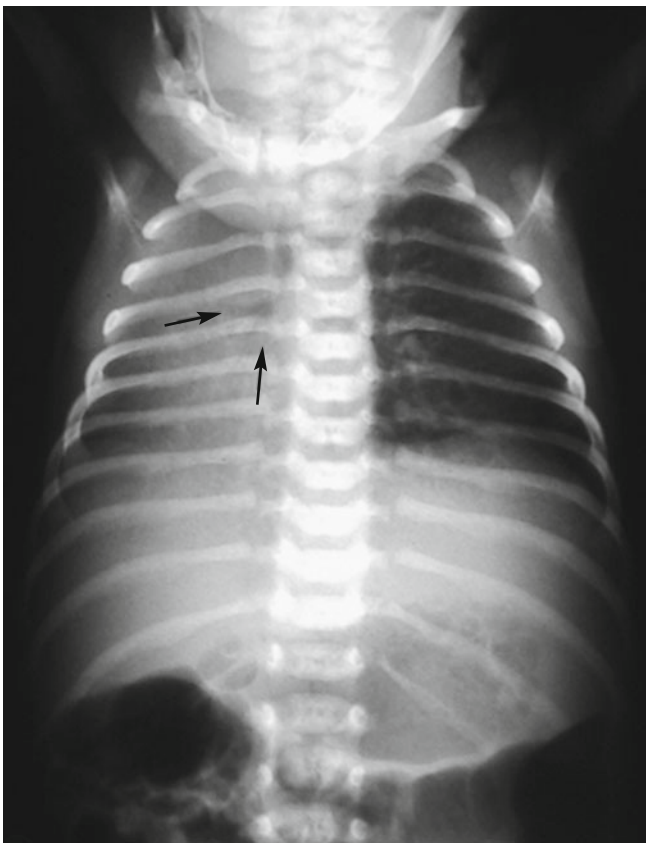

Fig. 56.9 Chest X-ray of a neonate with agenesis of the right lung. *Black arrows* show the right lobar bronchi ending blindly. Mediastinum shifts to the right side

occurrence, recurrent respiratory symptoms secondary to respiratory tract infections may appear later in life. On auscultation, mediastinal shift and hyperresonance of the contralateral hemithorax side are usually present. Asymmetry of the chest becomes more noticeable in the adult patient [61].

With postnatal compensatory growth the remaining lung often herniates into the contralateral chest. Chest X-ray shows a mediastinal shift towards the affected side (Fig. 56.9), and skeletal abnomalities may be present. Absent or incomplete lung development may be associated with abnormalities of the trachea, absence of one or both kidneys, cardiac defects (patent ductus arteriosus, patent foramen ovale, ventricular defects, pulmonary veins entering the azygos vein), gastrointestinal malformations (atresia ani, esophageal atresia, short bowel), skeletal anomalies (spina bifida, wedge-shaped vertebrae, hemivertebra, deformed ribs, absent left hand, absence of right radius, rudimentary atlas) deformed external ear, and many others. Differential diagnosis should be made with lung atelectasis, congenital diaphragmatic hernia, cystic adenomatoid malformation and pulmonary sequestration [62]. The difficulty of diagnosis depends on the side of the absent lung, the apparent dextrocardia induced by absence of the right lung being a striking clinical finding. Bronchoscopy has been used to establish the diagnosis of pulmonary agenesis. Typical findings include bronchus/i narrow ending blindly.

56.4.6.2 Prognosis

About 50% of patients survive; the mortality rate is higher with agenesis of the right lung than of the left lung.

56.4.7 Pulmonary Hypoplasia

Pulmonary hypoplasia (PH) is a common and serious problem in the perinatal period with a significant mortality rate. Premature rupture of membranes at less than 25 weeks' gestation, severe oligohydramnios (amniotic fluid index <4) for more than 2 weeks and earlier delivery are known risk factors for mortality [63]. Pulmonary hypoplasia is defined as a defective or incomplete development of lung tissue characterized by a reduction in the number of lung cells, airways and alveoli, resulting in a lower organ size and weight. It encompasses a wide spectrum of anatomic malformations ranging from total bronchial and parenchymal agenesis to mild pulmonary parenchymal hypoplasia. The lesions may be lobar, unilateral, or bilateral. Cases of isolated lobar defects have only been rarely reported. PH may be an isolated entity (primary PH) or be secondary to lesions restricting lung growth [64].

56.4.7.1 Etiology and Pathogenesis

Primary PH includes pulmonary agenesis (unilateral) [65] with or without associated malformations and idiopathic PH (bilateral). The causes of primary PH have not been identified. However, experimental models suggest that deficiencies in certain growth factors (e.g., epidermal growth factor and its receptor; mitogen-activated protein kinase, connective tissue growth factor) can result in disordered lung growth.

Nakamura and colleagues [66] revealed five statistically significant risk factors associated with secondary PH from a large series of autopsy cases: (1) hydrops fetalis; (2) renal anomalies; (3) diaphragmatic hernia; (4) skeletal anomalies; (5) oligohydramnios and polyhydramnios (Table 56.2).

Pathologically, the hypoplastic lung shows a low ratio of lung to body weight, low DNA content, or a decreased radial alveolar count [67]. Epithelial differentiation is delayed, and surfactant deficiency is associated [68]. Both pulmonary arterioles and bronchioles are decreased in size. Hypertrophy of the medial smooth muscle is a common finding in peripheral pulmonary blood vessels.

56.4.7.2 Clinical Aspects

In patients with PH, the clinical profile and the time of presentation vary depending on the extent of hypoplasia and other

Table 56.2 Causes of secondary pulmonary hypoplasia

Small fetal thoracic volume
- CDH
- Eventration of the diaphragm
- Abdominal mass lesions
- Exomphalos
- Pleural effusions with fetal hydrops
- Hydrothorax
- CAM
- Sequestration
- Malformations of the thorax (eg, asphyxiating thoracic dystrophy)
- Achondroplasia
- Thanatophoric dwarfism
- Osteogenesis imperfecta
- Thoracic neuroblastomas

Prolonged oligohydramnios
- Fetal renal agenesis
- Urinary tract obstruction
- Bilateral renal dysplasia
- Bilateral cystic kidneys
- Prolonged rupture of membranes

Early rupture of membranes
- More severe oligohydramnios (amniotic fluid index <4)
- Longer latent period before delivery

Decreased fetal breathing
- CNS lesions
- Lesions of the spinal cord, brain stem, and phrenic nerve
- Neuromuscular diseases (eg., myotonic dystrophy, spinal muscular atrophy)
- Arthrogryposis multiplex congenital
- Maternal depressant drugs

Congenital heart diseases with poor pulmonary blood flow
- Tetralogy of Fallot
- Hypoplastic right heart
- Pulmonary artery hypoplasia
- Scimitar syndrome causing a unilateral right-sided pulmonary hypoplasia

anomalies. Severe respiratory insufficiency resulting in a fatal outcome is the presenting symptom in a vast majority of preterm infants with severe bilateral PH. The history may include poor fetal movement or amniotic fluid leakage and oligohydramnios. In more mature neonates, mild to moderate hypoplasia or unilateral PH may be asymptomatic or may present with respiratory distress that requires mechanical ventilation. Newborn infants with moderate to severe PH often present with persistent pulmonary hypertension responding to inhaled nitric oxide [69] as a result of reduced vascular bed and secondary arterial muscular hypertrophy. Pneumothorax, spontaneous or associated with mechanical ventilation, may occur [70]. Compression deformities due to prolonged oligohydramnios, contractures, and arthrogryposis may be present when PH is associated with oligohydramnios. The Potter facies (hypertelorism, epicanthus, retrognathia, depressed nasal bridge, low set ears) suggest lung hypoplasia caused by the associated renal defects [71]. When the etiology of the hypoplasia is a neuromuscular disease, the patient may have myopathic facies, with a V-shaped mouth, muscle weakness, and growth retardation.

A number of studies have described associated anomalies with PH including heart defects (e.g., anomalous venous return to the right atrium or the inferior vena cava or scimitar syndrome, absence of the ipsilateral pulmonary artery), skeletal and vertebral anomalies (metacarpal and radial anomalies, failure of segmentation of thoracic or other vertebrae, rib abnormalities), abdominal defects (diaphragm, abdominal wall defects), facial abnormalities, as well as numerous lung and tracheo-bronchial tree defects. There have been a number of case reports of PH and tracheoesophageal fistula and a number of reports of an association of tracheal stenosis with pulmonary agenesis. Similar to patients with more severe degrees of PH, neonates with isolated lobar hypoplasia may demonstrate other congenital malformations.

In infants with unilateral pulmonary agenesis the diagnosis may be suspected by unilaterally decreased breath sounds and displacement of the mediastinum to the affected side. In right-sided hypoplasia, the heart is displaced to the right, which may lead to a mistaken diagnosis of dextrocardia. The external chest may appear normal or may be small and bell shaped, with or without scoliosis. In PH associated with congenital diaphragmatic hernia signs of respiratory distress are associated with scaphoid abdomen and bowel sounds upon chest auscultation. In older children, dyspnea and cyanosis may be present upon exertion, or a history of repeated respiratory infections may be noted.

56.4.7.3 Diagnostic Methods

The diagnosis of PH can be made with certainty only at autopsy by measurement of total lung DNA content. Because of this, the incidence of PH is probably underestimated. The diagnosis should be suspected in the presence of perinatal factors suggestive of PH, unexpected respiratory distress, specific findings at clinical examination.

Diagnosis of unilateral lobar PH requires a high index of clinical suspicion and experience with reading neonatal and infant radiographs. Chest CT or MRI may be needed to better delineate the defect and to detect possible associated defects (accessory diaphragm, pulmonary sequestration adjacent to a small diaphragmatic hernia). Unilateral PH should be suspected in the presence of hyperlucency and smallness of one lung. Hyperdistention of the contralateral lung can be seen.

The diagnosis of primary PH implies a lack of any specific pathophysiologic process that could cause fetal lung compression. For the diagnosis of secondary PH abdominal masses, such as cystic renal diseases and an enlarged bladder, must be sought. Associated anomalies of the cardiovascular, gastrointestinal (e.g., tracheoesophageal fistula, imperforate anus, communicating bronchopulmonary foregut malformation), and genitourinary systems, as well as skeletal anomalies of the vertebrae, thoracic cage, and upper limbs, may be found upon examination.

Echocardiography is fundamental to reveal possible cardiac defects. Prenatal ultrasound [72] and, more recently, fetal MRI constitute the methods of choice to investigate the fetal lung development throughout pregnancy [73].

Basically, the MR assessment of the fetal lung growth and the maturation consists of three diagnostic elements. First, fetal MR volumetry is used to identify restricted and insufficient lung growth. Second, the evaluation of signal intensities using different MR sequences provides information about the maturation of the fetal lung. Third, the high resolution and tissue contrast of MR allows us to examine the structure of the fetal lung and to offer a more detailed diagnosis of fetal pulmonary pathologies [74].

56.4.7.4 Differential Diagnosis

The differential diagnosis of respiratory distress in the newborn associated with marked opacification of one side of the thorax on radiograph includes atelectasis, congenital diaphragmatic hernia, congenital cystic adenomatoid malformation, pulmonary sequestration, chylothorax, PH, bronchogenic cyst, and a chest tumor (e.g., neuroblastoma, teratoma, fibrosarcoma). In a right-sided CDH, there may be opacification of the right hemithorax if the liver is occupying that space. A left-sided CDH will have air-filled loops of bowel in the chest except possibly in a chest radiograph taken shortly after birth or following bowel decompression. Congenital cystic adenomatoid malformation will usually appear as a cystic mass rather than a homogenous opacification. Obstruction of the bronchus with resultant opacification of the hemithorax may occur with a bronchogenic cyst or a vascular sling. Neonatal chest tumors are very rare and may present as a focal abnormality on chest X-ray.

In cases of bilateral PH with hyperlucent lungs idiopathic persistent pulmonary hypertension should be considered in the differential diagnosis, whereas the presence of small lungs may suggest spinal thoracic dysplasia or neuromuscolar disesases.

56.4.7.5 Therapy and Treatments

Treatment is largely supportive, and prognosis will depend on the presence or absence of the other anomalies. Inhaled nitric oxide (iNO) administered to preterm infants with prolonged premature rupture of membranes (PPROM), oligohydramnios, and PH improves oxygenation, survival, or other clinical outcomes [75]. Because of the surfactant deficiency a significant number of infants with PH require surfactant replacement therapy and high-frequency ventilation [76].

Conventional therapies may fail in these infants who then become candidates for ECMO [77]. The eventual survival is limited by the underlying degree of the PH.

Fetoscopic temporary tracheal occlusion (FETO) may improve outcome in poor-prognosis fetuses with CDH [78].

56.4.7.6 Prognosis

Long-term consequences of PH include: reduced exercise tolerance, even in mild cases, scoliosis in adolescent years, recurrent respiratory infections, chronic pulmonary insufficiency. Right-sided hypoplasia has a worse prognosis than left-sided hypoplasia, probably because of the loss of the bigger right lung mass and more severe mediastinal shift and great vessel displacement [79].

During the first 48 hours of life, the best oxygenation index above 13 and the best $PaCO_2$ above 45 mmHg were predictive of poor outcome [80].

56.4.8 Oligohydramnios Syndrome

Bilateral PH commonly occurs in association with oligohydramnios caused by either renal disease in the fetus or chronic leakage of amniotic fluid as a consequence of PPROM.

For the fetal lung to develop normally, the fetal airways must be filled with fluid [81]. Active chloride transport by epithelial cells results in passive water movement into the fetal airspaces, with a net production rate of lung fluid of about 4–5 mL/kg/h.

The pressure in the fetal trachea exceeds that in the amniotic fluid by about 2 mmHg, generating an outflow resistance that maintains the fetal lung fluid in the fetal lung. Several factors affect the volume and composition of the amniotic fluid, including amount of fetal urine and chronic leakage of amniotic fluid secondary to premature rupture of membranes at 15–28 weeks' gestation.

Any alteration in the critical volume and pressure relationships of amniotic fluid and fetal lung fluid during the canalicular stage of fetal lung development can induce hypoplasia [82].

Infants subject to oligohydramnios resulting from chronic leakage of amniotic fluid over many weeks may exhibit a varying degree of PH. Oligohydramnios may act by compressing the fetal thorax or altering the dynamics of lung fluid so that full expansion of the lungs cannot be maintained. The severity of the PH is directly proportional to rupture of the membranes early in gestation, longer duration of rupture, and the presence of the oligohydramnios during the period of rupture. Oligohydramnios can be associated with a spectrum of a fetal positional and compression abnormalities, such as pes equinovarus and a bell-shaped thorax.

The presence of PH in infants with bilateral renal agenesis was first recognized by Potter in 1946. It has since become apparent that a similar picture of the lethal PH is seen in conjunction with bilaterally dysplastic kidneys with or

without cyst formation and in infants with congenital obstructive uropathy. In the cases of renal agenesis the reduction in lung volume and number of the alveoli is greater than in renal dysplasia, where these changes are more variable in their severity [83].

Despite attempts at in utero decompression of the urinary system, fetal surgery in this disease remains controversial and has met with limited success.

56.4.9 Congenital Pulmonary Lymphangiectasia

Congenital pulmonary lymphangiectasia (CPL) is a rare developmental disorder involving the lung and is characterized by pulmonary subpleural, interlobar, perivascular, and peribronchial lymphatic dilatation.

Reports of occurrence in siblings suggest a genetic inheritance in some families and possibly an autosomal recessive mode of transmission [84]; however most cases occur sporadically [85]. Male are more frequently affected than females (ratio 2:1). The incidence of CPL is not clearly defined. Autopsy studies suggest that approximately 0.5–1% of infants who are stillborn or die in the neonatal period have CPL [86].

56.4.9.1 Etiology and Pathogenesis

Noonan and colleagues classified CPL into three patho-physiological groups [87]:

- Type 1 - Generalized lymphangiectasis with thoracic and extrathoracic involvement.
- Type 2 - Lymphangiectasis secondary to pulmonary venous obstruction due to congenital heart disease.
- Type 3 - Primary pulmonary developmental defect of the lung.

Clinically, two forms of CLP have been recognized: primary or congenital (type 1 and 3) and secondary (type 2), which occurs as a result of injury to the lymphatic vessels. The primary type has a neonatal onset and is often fatal [88]. It may be caused by a congenital defect in the primary development of the lung, or may represent the localized expression of more generalized lymphatic involvement (non immune hydrops).

The pulmonary lymphatic system is normally well developed by the end of the 14th week of gestation. Initially, large lymph channels are present in the normal fetal lungs, which later undergo spontaneous regression. It is believed that failure of these channels to undergo the normal regression leads to primary pulmonary lymphangiectasia.

The secondary form results from impaired lymphatic drainage and increased lymphatic production as a consequence of increased venous and lymphatic hydrostatic pressure; it may also be due to cardiac lesions, which have been hypothesized to interfere with the normal regression of the lymphatic tissue elements after the 16th week of fetal life [88]. Hypoplastic left heart syndrome, pulmonary vein atresia, congenital mitral stenosis, cor triatum, and thoracic duct agenesis are the most likely causes of secondary CPL.

Congenital PL may be associated with non-immune hydrops fetalis and with congenital chylothorax [89].

56.4.9.2 Clinical Aspects

Clinical diagnosis of CPL can be strongly suspected in neonates who present severe respiratory distress with or without generalized or localized lymphedema, and with pleural effusion (especially if chylous) either monolateral or bilateral although the disease affects both lungs.

In the post-neonatal period, CPL can occur any time in childhood or even adult life. During both the neonatal and post-neonatal period, CPL may be associated with chylothorax, chylopericardium, and chylous ascites [90]. In older children it is frequently associated with recurrent cough, wheeze, increased respiratory effort with inspiratory crackle, and even congestive heart failure [91].

CPL has been described in patients with 46,XY/46,XX mosaicism, ichthyosis congenita, Noonan syndrome, Turner syndrome, Fryns syndrome, Down syndrome, and other syndromes that suggest genetic predisposition [88].

Diagnostic Methods

During the prenatal period, all causes leading to hydrops fetalis have to be taken into consideration. The diagnostic approach includes the following: complete family and obstetric history, laboratory investigations including blood type, Rhesus factor (Rh), antibody screening, TORCHES-CLAP titer (*Toxoplasma gondii*; Rubella virus; Cytomegalovirus; Herpes simplex virus; Enterovirus; Syphilis; Varicella-zoster virus; Lyme disease; AIDS; Parvovirus B19), metabolic studies, and hemoglobin (Hb) electrophoresis [92]. An instrumental evaluation is needed to rule out various conditions possibly related to CPL and to establish whether CPL is primary or secondary.

Diagnostic methods that may be useful in evaluating CPL include conventional radiologic studies, echocardiography, high-resolution CT [93] and MR imaging [94], lymphoscintigraphy [95], lung functionality tests [96], lung biopsy [97], bronchoscopy, and pleural effusion examination [98].

Chest X-rays usually show hyperinflation with interstitial markings. CT demonstrates diffuse thickening of the interstitium, both of the peribronchovascular interstitium and the septa surrounding the lobules. Coronal MRI T1 may show thickening of the interstitium, pleural fluid effusion, and atelectasia, if present. Axial MRI T2 usually shows high-signal material within the pulmonary interstitium, which is very often associated with pleural effusion. Despite the greater

dose of radiation that is given during CT as compared to chest radiography, CT is preferable for the diagnosis of CPL and, more generally, for the diagnosis of pediatric interstitial lung disease. Lymphoscintigraphy is a mildly invasive technique that provides valuable morpho-functional information regarding the lymphatic system [99]. It highlights the accumulation of lymphatic fluid in the interstitial tissue that causes swelling, which is most evident in the limbs.

In the few cases in which lung function tests were performed, they showed various patterns including restrictive, obstructive, and normal values. It is noteworthy that pulmonary function tests were stable over time in the patients who obtained multiple values.

Bronchoscopic evaluation, while not specifically indicated in CPL, may be useful for ruling out other pulmonary pathologies. No tracheo-bronchial anatomical abnormalities were reported in CPL patients who were evaluated by bronchoscopy.

Lung biopsy may be useful to demonstrate the presence of dilated lymphatic spaces in the sub-pleural connective tissue, along the thickened interlobar septa, and around the bronchovascular axes. Great care must be taken when interpreting lung biopsies. In fact, the pathological findings in CPL patients may change a great deal over time, especially in case of viral infection, and, more generally, may range from initial recognition of minimal evidence of lymphatic dilatation to proof of severe lymphangiectasia. In this case, the lymphatic vessels are characterized by a thin wall, devoid of smooth muscle, and with slightly dilated lumen, lined by flattened endothelial cells.

56.4.9.3 Differential Diagnosis

During the neonatal period, transient tachypnea of the newborn, pulmonary aspiration syndrome and interstitial pulmonary infection are well known and are usually taken into consideration in the differential diagnosis of respiratory distress syndrome in the neonate.

Rarer conditions must also be considered in the differential diagnosis of chronic interstitial lung disease in infants, and include surfactant protein B deficiency, desquamative interstitial pneumonitis (familial and non-familial forms), pulmonary alveolar proteinosis, idiopathic pulmonary fibrosis, lymphoid interstitial pneumonitis, cellular interstitial pneumonitis, and chronic pneumonitis of infancy. Moreover, other conditions that can mimic interstitial lung disease in infants and children should be considered. These include neuroendocrine cell hyperplasia of infancy, acute pulmonary hemorrhage of infancy, follicular bronchiolitis, pulmonary vascular disorders (obstructive pulmonary venous disease, i.e., total and partial anomalous pulmonary venous return, pulmonary vein atresia or stenosis), hereditary hemorrhagic teleangiectasia, pulmonary hemangiomatosis, various systemic diseases, and metabolic lipid storage disorders.

56.4.9.4 Therapy and Treatments

Delivery room management could be a challenge in the presence of severe respiratory distress associated with pleural effusion, and multiple procedures might be required. Tracheal intubation and assisted ventilation are usually necessary. Infants with persistent pulmonary hypertension and difficulties oxygenating should be ventilated with high-frequency oscillation. This type of ventilation could be useful in recruitment of alveoli and will decrease barotrauma and volutrauma. Higher sustained mean airway pressure may also favor alveolar lymph drainage. Thoracentesis is useful in cases with conspicuous pleural effusion. Some patients with CPL could benefit from inhaled nitric oxide (iNO). Patients with acute respiratory failure who are refractory to maximal conservative treatment might be considered candidates for ECMO [100].

56.4.9.5 Prognosis

Recent advances in neonatal intensive care have changed the previously nearly fatal outcome of PL at birth. However, contradictory data have been reported regarding the outcome and do not allow a consistent prognosis to be established. The outcome is more likely to be favorable if there are no associated defects. Respiratory problems can continue over the first years of life and often require home supplemental oxygen and symptomatic treatment for recurrent cough and wheeze.

56.4.10 Chylothorax

Chylothorax is an unusual entity in the neonate that commonly is associated with respiratory symptoms in the first day of life and may be expression of CPL. The incidence of congenital chylothorax is about 1:10,000–15,000 pregnancies, with a male-female ratio of 2:1. Chylothorax presenting at birth may be associated with cardiac anomalies, various syndromes, thoracic ductal or venous thrombosis, birth trauma or local compression of the thoracic duct, congenital malformation of the lymphatic system (lymphangiomatosis) [86, 101, 102]. Chylothorax has been described as a complication of Down syndrome, in which there is presumably a significant maldevelopment of the lymphatic system. Other patients may have Turner or Noonan syndrome. Infants may have cystic hygroma malformations [86, 103]. Acquired chylothorax results from damage to the thoracic duct. It has been reported as a surgical complication in the repair of congenital diaphragmatic hernia, tracheoesophageal fistula, and a variety of congenital heart disorder (e.g., PDA ligation). Acquired chylothorax may also be the consequence of drainage of pneumothorax when the tube is inserted too far [104]. In many cases, however, no underlying cause for the chylothorax is found. Chylothorax is

more common on the right side: Chernick and Reed said that 53% of the cases were on the right side, 35% on the left side, and 12% were bilateral [104]. If the problem occurs early in the fetal life, infants may have significant pulmonary hypoplasia [105]. Since chylothorax is typically unilateral, there may be decreased breath sounds over the side of the effusion and mediastinal shift to the contralateral side. Bilateral chylothoraces should be considered in the differential diagnosis for any infant who cannot be ventilated in the delivery room. The characteristic roentgenographic appearance is similar to that of a large pleural effusion depressing the adjacent diaphragm and displacing the mediastinum.

The evaluation of the pleural effusion is essential for the diagnosis of chylothorax. Chylothorax is usually diagnosed in the presence of "milky" pleural effusion with a triglyceride level >1.1 mmol/L and a cell count >1000 cells/μL, with a predominance of lymphocytes (approximately 80%), according to the criteria drawn up in previous reports (Table 56.3) [98]. However, this is an unreliable diagnostic test in malnourished patients and in patients not receiving enteral nutrition, including the fetus and occasionally the neonate. Without enteral feeding, not enough chylomicron (the main triglyceride carrier) is produced to raise chyle triglyceride levels. In these patients, a diagnosis of chylothorax may easily be made by detecting lymphocytes in the pleural fluid.

Treatment of chylothorax may require repeated thoracenteses or even thoracostomy tube drainage to prevent respiratory failure. The large amount of fluid that is drained over days and weeks leads to the loss of great quantities of albumin, immunoglobulin, and many other plasma factors that must be replaced, in some cases even on a daily basis. Once drainage is accomplished, these infants are placed on formulas containing medium-chain triglycerides (MCTs) to reduce thoracic duct lymph flow. Oral intake of protein and water also stimulates thoracic duct lymph flow, so in resistant cases the infant is managed with nothing by mouth, with nutritional support through a central line [106].

Octreotide and antiplasmin have been used in CPL and chylothorax [107].

A variety of approaches have been used with some success to treat the infant with persistent chylothorax, including direct attempts at repair, patching with fibrin glue, and obliteration of the pleural space with sclerosing agents [108]. Congenital chylothorax also has been managed successfully using a pleuroperitoneal shunt [109]. Finally some reports suggest that ligation of the thoracic duct below the area of leakage is highly effective; this procedure is well tolerated without accumulation of fluid in the peripheral tissues or in the peritoneum [110].

Table 56.3 Composition of chyle [99]

Measurement	Mean	Range
Total protein (g/dL)	3.56	1.89–6.17
Albumin (g/dL)	2.24	1.26–3.0
Total lipids (mg/dL)	1180	56–3500
Cholesterol (mg/dL)	81	48–200
Triglycerids (mg/dL)	197	123–234
White blood cells (/mm^3)	15.200	0–29.000
Lymphocytes (%)	90	70–100
pH	7.5	7.4–7.8
Specific gravity	1.013	1.008–1.027

56.4.11 Misalignment of Pulmonary Veins with Alveolar Capillary Dysplasia

Alveolar capillary dysplasia (ACD) is a rare disorder typically presenting at birth with severe hypoxemia, although an unusual late onset has been reported at 6 weeks of life in an infant with a patchy distribution of disease [111]. No sex predilection has been identified. While the cause is unknown, there have been six cases reported in siblings, indicating that in some cases this may be a familial disorder with autosomal recessive inheritance [112].

56.4.11.1 Etiology and Pathogenesis

Pathological features include a paucity of alveolar capillaries, widened alveolar septae, and increased muscularization of pulmonary arterioles [113]. There is usually malpositioning ("misalignment") of pulmonary veins in the bronchovascular bundle, but this is not required for the diagnosis. A focal distribution of disease has been described, which makes it necessary to examine multiple lung sections if ACD is suspected [114]. ACD is characterized by failure of formation of alveolar capillaries, poor capillary apposition and density, together with medial arterial hypertrophy and misalignment of pulmonary vessels. Airspaces are lined by simple cuboidal cells, and there is a striking lack of proximity of capillaries and pneumocytes. Pulmonary veins are present in the bronchovascular bundle rather than in their normal position in the interlobular septa.

56.4.11.2 Clinical Aspects

The initial presentation is identical to severe idiopathic pulmonary hypertension of the newborn [115]. However, infants with ACD do not respond, or respond only transiently to therapies that are usually effective in reversing pulmonary hypertension.

The diagnosis of ACD should be considered in infants who present with severe hypoxemia and idiopathic pulmonary hypertension, and who do not respond appropriately after 7–10 days of standard treatment as described below. The majority of patients with ACD (approximately 75%) will have other associated anomalies of the cardiovascular,

gastrointestinal, urogenital, or musculoskeletal systems [116–118]. The initial chest radiograph is usually normal. The diagnosis can only be confirmed by lung biopsy or autopsy.

56.4.11.3 Differential Diagnosis

Conditions that must considered in the differential diagnosis of ACD are idiopathic persistent pulmonary hypertension of the newborn and surfactant protein B deficiency [119].

56.4.11.4 Therapy and Treatments

Standard therapies include mechanical ventilation, high concentrations of inspired oxygen, iNO and ECMO support [120]. These therapies prolong life by days to weeks, but have not led to long-term survival [121]. Theoretically, ACD could be treated by lung transplantation. However, successful transplantation has not yet been reported. Donor availability continues to limit the utilization of lung transplantation for neonatal diseases.

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57

Neonatal Pulmonary Physiology of Term and Preterm Newborns

Corrado Moretti and Paola Papoff

57.1 Introduction

The transfer of O_2 to the tissues and the removal of CO_2 are guaranteed by the simultaneous activity of the respiratory apparatus and of the cardio-circulatory system which ensures that O_2 reaches the tissues and that the CO_2 produced by cell metabolism is removed [1].

The main steps in this process are:

- pulmonary ventilation, which consists in the rhythmic introduction of fresh gases into the alveolus; tidal volume is a term deriving from "tide" which offers an excellent representation of the flow and reflow of the gases;
- the diffusion of O_2 and CO_2 at the alveolus-capillary interface;
- capillary perfusion, whereby O_2 and CO_2 are transported into and out of the tissues.

Unlike the respiratory apparatus, the cardio-circulatory system is a closed-loop system with a one-way flow.

57.2 Lung Mechanics

Alveolar ventilation is obtained through pulmonary expansion, which in turn occurs through dilatation of the ribcage. At rest, the lungs and ribcage are in a situation of equilibrium determined by the resultant of two contrasting forces: elastic inward recoil of the pulmonary parenchyma and outward elastic traction of the ribcage. The pulmonary volume that results from this equilibrium is defined as functional residual capacity (FRC).

The consequence of this interaction in opposite directions is the presence of a negative pressure of around –3, –5 cm H_2O in the intra-pleural space, which is a virtual cavity. During normal breathing, most of the tidal volume is ensured by the diaphragm, a muscle supplied by the phrenic nerves, whose contraction causes an increase in the vertical diameter of the ribcage; the increase in the antero-posterior diameter is due to the contraction of the internal intercostal muscles whereby the ribs rotate upwards along their axis.

Dilatation of the ribcage during inspiration causes a reduction in intra-pleural pressure, and the lungs – hence the alveoli – undergo passive expansion [2]. The decrease in intra-alveolar pressure produces an air flow in the respiratory system; the flow stops at the end of inspiration when the alveolar pressure returns to a state of equilibrium with atmospheric pressure (Fig. 57.1).

In newborns, chest stability is facilitated by a relatively horizontal position of the ribs so that the intercostal muscles, which contract simultaneously with the diaphragm, can provide structural stiffness for the ribcage rather than expanding it during inspiration. Inspiration and expiration are therefore ensured above all by the activity of the diaphragm, which is less efficient in newborns compared to adults due both to its rather flat conformation that reduces its excursion and to its histological characteristics. In premature infants, in particular, the proportion of diaphragmatic slow-twitch muscle fibers that are resistant to oxidation and fatigue (Type I) is much smaller (10%) than the fast-twitch fibers which tire more easily (Type II); the proportion of Type I fibers increases progressively with age (about 25% in a term infant and 55% at eight months) [3].

Moreover, the chest of a premature infant is subject to distortion owing to the poor ossification of the ribs and sternum and because the intercostal muscles are not fully functional. The inward distortion of the chest during inspiration compels the diaphragm to do more work: indeed, in order to maintain the tidal volume constant, the excursion of the diaphragm must increase so as to offset the paradoxical movement of the ribcage. This phenomenon contributes to accelerating the respiratory functional exhaustion to which low birth-weight infants with respiratory distress are subject to.

The expiratory phase, by contrast, is usually a passive phenomenon determined by the forces of elastic recoil of

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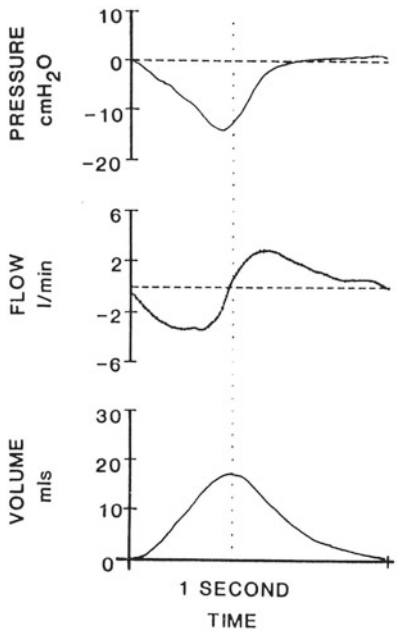

Fig. 57.1 Pressure, air flow, and volume signals of a respiratory cycle. Reproduced from [4], with permission

distended tissues: the greater the expansion of the lungs during inspiration, the greater its elastic recoil. The intervention of expiration muscles, the most important ones being the muscles of the abdominal wall, occurs only in some physiological situations such as coughing and sneezing or in some pathological conditions.

57.3 Elastic Properties of the Lungs

Compliance (C) is a value that expresses the elasticity of the lung or, rather, its capacity to be distended. It is defined by correlating the pressure applied to the alveoli (P) to the ensuing volume variations (V):

$$C = V/P$$

Compliance is usually represented through pressure/volume curves (Fig. 57.2): the higher its value the greater the variation of the amount of gas in the lung per unit pressure and the steeper the slope of the curve. A peculiar characteristic of lung pressure/volume curves is that inflation and deflation have different pressure curves, a phenomenon that is called hysteresis.

The factors determining the degree of elasticity of the lung are the presence of elastic fibers in the parenchyma, and the surface tension forces of the liquid that wets the alveoli walls [5]. Surface tension forces are generated by the cohesion between the molecules making up the liquid: these forces are balanced in the liquid phase but not at the air-liquid interface; the result is that the molecules on the surface exercise greater attraction towards the surrounding molecules. The importance of the role of the surface tension forces may be demonstrated through a simple experiment: by expanding an isolated lung with saline solution, and then with air, two different pressure/volume curves are obtained. In the first case the recoil forces are entirely due to the elastic properties of the lung tissue because there is no air-liquid interface; by contrast, when the lung is expanded with gas, the surface tension forces require a greater pressure to obtain the same expansion; moreover, it must be pointed out that when the lungs are filled with saline solution the hysteresis disappears.

The presence on the surface of the alveoli of a tensioactive factor or surfactant, consisting of various phospholipids, has the function of reducing the superficial tension forces and of stabilizing the size of the alveoli.

According to Laplace's law

$$\text{Pressure} = 2 \times (\text{surface tension})/\text{radius}$$

the pressure required to maintain the alveoli expanded increases proportionally to the decrease in its radius: the consequence should be that the smaller alveoli empty out into the larger ones, but the action of the surfactant, i.e., decreasing surface tension, is more effective as the alveolus decreases its size as a result of the greater concentration of surfactant molecules. The intra-alveolar pressure is thus uniformly maintained in the pulmonary parenchyma and the size of the alveolus is kept stable.

The effects of the surfactant on lung mechanics can be easily understood from the analysis of Fig. 57.3, which shows two pressure/volume curves obtained by inflating a lung affected by respiratory distress syndrome (RDS), before and after treatment with surfactant.

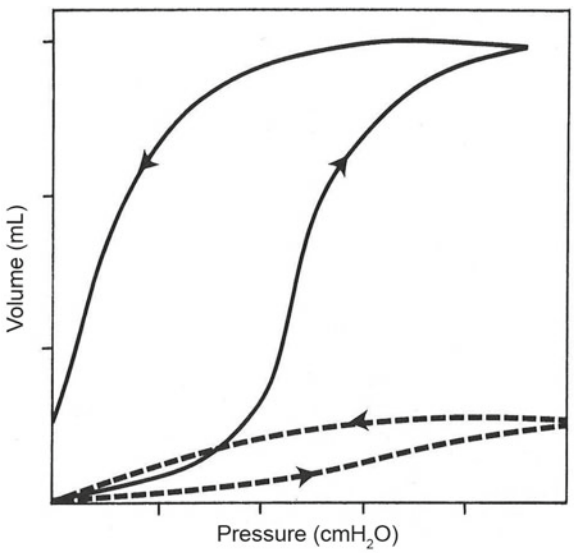

Fig. 57.2 Pressure/volume diagram in a neonate with RDS (*dashed line*) and in a normal neonate (*solid line*)

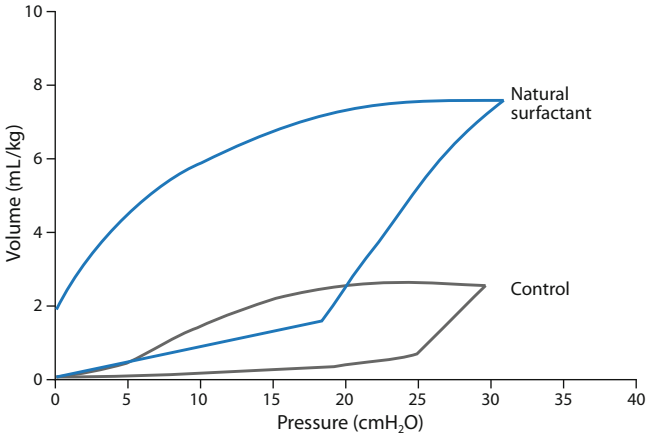

Fig. 57.3 Pressure-volume relationships for the inflation and deflation of surfactant-deficient and surfactant-treated preterm rabbit lungs. Modified from [6]

The fundamental differences between the two curves are:

- the opening pressure, i.e., the pressure at which the alveoli begin to expand, is higher before being treated with surfactant;
- lung expansion at the same peak pressure value is better after treatment with surfactant;
- before treatment with surfactant the lung does not have stability in the expiratory phase and has a tendency to collapse and to lose FRC.

In summary, the surfactant has the following functions:

- it reduces the respiratory workload since the lung is more prone to distension;
- it increases the FRC given the lesser tendency of the lung to collapse;
- it has an anti-edema role since the increase in surface tension facilitates the attraction of liquids from the capillaries into the alveoli;
- it stabilizes the sizes of the alveoli.

A further factor that contributes to stabilizing the sizes of the alveoli is their mechanical interdependence: if an alveolus tends to collapse, the stress on the walls of the adjacent alveoli increases and this helps it remain expanded. The alveoli support each other thus contrasting any tendency towards a reduction or increase in volume of any single alveolus.

57.4 Airway Resistance

The forces that have to be overcome in order to ventilate the air inside and outside the respiratory system are not only the forces of elastic recoil of the lungs and chest but also the airway resistance.

The forces required to ensure breathing, whether the energy spent is stored and retrieved thereafter, or whether it is lost by the system in the form of heat, are defined conservative forces or dissipation forces. Conservative forces are the (volume-dependent) pressures generated by the elastic properties of the lungs and of the rib-cage, forces that tend to cause these structures to return to their initial position after a deformation: the energy spent on overcoming these forces during inspiration is recovered during expiration, which is a passive phenomenon. Conversely, the dissipation forces are the (flow-dependent) energies required to overcome not only the friction of the gases against the airway walls, but also the viscoelastic properties of the respiratory system determined by the molecular movements in the tissues. The dissipation forces are also defined as resistance forces: the energy required to overcome these forces is lost in the form of heat and hence can no longer be used.

In lung physiology the term airway resistance (R) is used to define the ratio between the difference in pressure between the alveoli and the mouth (ΔP) and the resulting flow (V):

$$R\ (cmH_2O/L/sec) = \Delta P\ (cmH_2O)/V\ (L/sec)$$

In the respiratory system some 30–40% of the resistance occurs at the nose, in the oro-pharynx and in the pharynx, while at the level of the trachea and bronchial tree the structures that resist most against the flow are the average sized bronchi and not the smaller sized airways.

The airways can indeed be schematized as a series of successive dichotomies (22 generations or bronchial branching): at each branch division the size of the segments slightly decrease in size, but since the number of elements doubles, the total cross section area increases with respect to the previous level (Fig. 57.4). Thanks to this architecture the resistance of the peripheral airways is very mild even if the flow runs along ducts whose size is truly very small.

The factors that determine the degree of resistance (R) of a duct include its length (l), its radius (r) and type of flow, i.e., laminar or turbulent flow

$$R = l/r^4$$

Turbulent flow, which occurs mainly at the points of branching or when the air flow is high and the radius of the duct is large, generates a larger resistance compared to laminar flow. The pressures in a laminar flow are proportional to the flow rate whereas in a turbulent flow they are proportionate to the square of the flow; in these conditions the energy needed is obviously greater. The progressive increase in total cross section area towards the periphery of the lung implies a progressive reduction in the linear velocity (linear velocity = air flow/cross section area) of the air flow, which is quite low and of the laminar type at the level of the smaller airways, whereas in the trachea and in the larger branches it is essentially turbulent.

Lung volume is another important factor for the resistance of the airways, above all of those with little or no cartilage structure. Indeed the small airways distend and compress very

easily, and the transmural pressure gradient of the walls of the small airways is important in defining their radius: during deep inspiration the intrapleural negative pressure increases thus causing an increase in the transmural pressure gradient and hence an increase in their size. A further factor consists in increasing the elastic tension exercised by the pulmonary parenchyma on the airways during volume expansion in the inspiration phase. The resistance of the airways therefore is less during inspiration as compared to expiration; in addition, during forced expiration, as occurs in the newborn when he cries, resistance is influenced by the dynamic compression of the airways.

Making the intrapleural pressure positive determines changes in transmural pressure whose effects are clearly seen in Fig. 57.5, which represents the pressure values that come into play during normal expiration and forced expiration. During forced expiration the pressure inside the peripheral airways is greater than the intrapleural pressure thanks to the pulmonary elastic recoil forces; in the larger airways by contrast, the pressure comes progressively closer to atmospheric pressure and is hence lower than intrapleural pressure. The point at which the pressure inside and outside the airways is equal is defined as equal pressure point (EPP), situated in normal individuals in the central airways within the first five branchings of the bronchial tree. Downstream from this point the airways are compressed and the compression increases with greater expiration effort, which will thus not be capable of increasing the flow velocity (dynamic flow limitation).

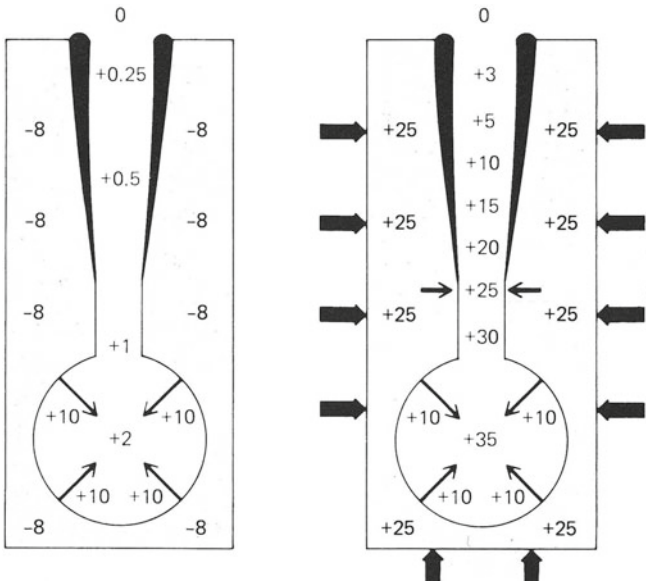

Fig. 57.5 Dynamic compression of airways. On the left, passive expiration: intrapleural pressure is –8 cm H_2O, pulmonary elastic recoil forces pressure is + 10 cm H_2O and alveolar pressure is +2 cm H_2O. On the right, forced expiration: intrapleural pressure is +25 cm H_2O, pulmonary elastic recoil forces pressure is +10 cm H_2O and alveolar pressure is +35 cm H_2O. Reproduced from [4], with permission

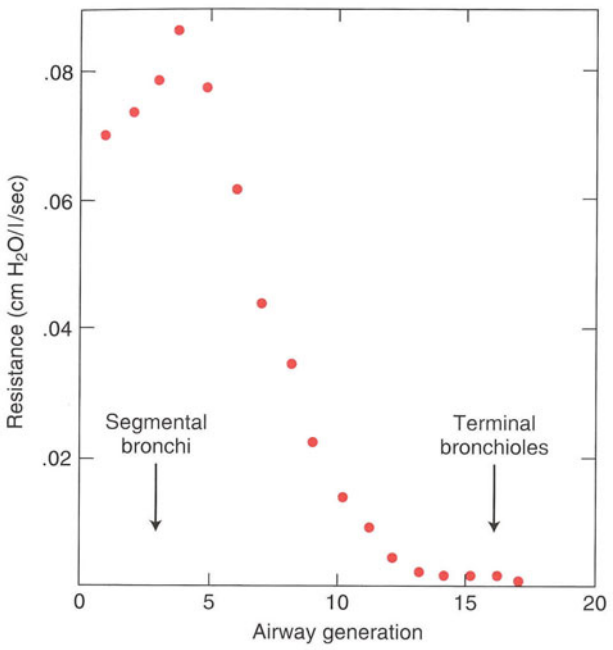

Fig. 57.4 Location of the main site of airway resistance. Note that the intermediate-sized bronchi contribute most of the resistance and that relatively little is located in the very small airways. Reproduced from [7], with permission

The expiratory flow also decreases with the progressive reduction of the pulmonary volume for the attenuation of the elastic recoil forces and for the smaller size of the airways; as expiration proceeds, the EPP moves distally towards the inside of the lung. The flow limiting mechanism is enhanced in some pathologic conditions characterized by an increase in peripheral resistance because the pressure drop is amplified inside the airways, or in others marked by a pressure reduction of the elastic component as occurs in emphysema.

57.5 Alveolar Ventilation

The amount of fresh air ventilated into the airways in one minute, or minute volume $\dot{V}_E$ ($\dot{V}$ = volume/time unit; the minute volume is measured with the amount of expired gas and it is indicated with $\dot{V}_E$), corresponds to the tidal volume (V_T) times the respiratory rate (RR):

$$\dot{V}_E = V_T \times RR$$

The tidal volume, that is to say the inspiration and expiration volume for each normal breathing cycle, measures about 6–8 mL/kg in the newborn, and 4–6 mL/kg in a preterm infant; about 30% of this volume remains inside the airways without taking part in the exchange of gases with the blood (anatomic dead space) and is called the dead space volume ($\dot{V}_D$). Alveolar ventilation ($\dot{V}_A$) is the volume of fresh air that

enters the respiratory zone every minute and is actually available for the exchange of gases; the $\dot{V}_A$ corresponds to:

$$\dot{V}_A = \dot{V}_E - \dot{V}_D$$

A reduction in the expansion of the lung, typical of RDS, causes a drop in V_T and hence in alveolar ventilation since the dead space remains unchanged; this compels the newborn to increase respiratory rate to keep the minute volume constant.

In physiological conditions the amount of air that remains inside the lung at the end of normal expiration, or functional residual capacity, is around 20–30 mL/kg; therefore, for each breathing cycle, only a part of the air in the alveoli (around 1/5–1/6) will be replaced with new air from the atmosphere. This slow renewal of air means that the variation in gas concentration in the alveoli is only 3–4 mmHg for each breathing cycle; relative constancy in gas concentration in arterial blood therefore depends on the buffer action of the FRC.

Let us now analyze the partial gas pressures in an ideal lung, considering that barometric pressure is around 760 mmHg: 20.9% of it is generated by O_2 and 79% by nitrogen; as a consequence, the PO_2 in the air is 20.9% of 760, that is 159 mmHg. The air inspired is saturated with aqueous vapor in amounts proportionate to body temperature; the value of the partial pressure of aqueous vapor at 37°C is 47 mmHg, which is to be subtracted from the relative value at total pressure of unsaturated air. The PO_2 of inspired air is hence 20.9% of 760 – 47 mmHg, therefore around 149 mmHg. The alveolar PO_2 (PAO_2) is less than that of the inspired air because the alveolar air is continuously impoverished of O_2 and enriched with CO_2 and its value is around 100 mmHg (as shown in Fig. 57.10a). The partial pressure values of O_2 and CO_2 at the alveoli are determined by the balance between alveolar ventilation, consumption of O_2 and production of CO_2.

Alveolar ventilation is regulated by the respiratory centers in such a way as to maintain the value of the arterial PCO_2 ($PaCO_2$) around 40 mmHg and the value of the arterial PO_2 (PaO_2) around 100 mmHg [8]. The value of arterial PCO_2, which is normally identical to the alveolar PCO_2 ($PACO_2$), is inversely proportionate to the degree of ventilation and is hence used as an index of alveolar ventilation; if alveolar ventilation is halved, the $PaCO_2$ doubles, if alveolar ventilation doubles, the $PaCO_2$ is halved (Fig. 57.6). Also the PaO_2 variations are proportionate to the degree of ventilation and inversely proportionate to the $PaCO_2$, in that the total sum of the partial pressures of the gases at the alveoli is always 760 mmHg. Actually, the partial pressure of aqueous vapor does not vary, and during ventilation the partial pressure of nitrogen varies slightly. However, the PaO_2 is not used as an index of ventilation because, unlike the $PaCO_2$, its value varies also as a function of the presence of an intra or extrapulmonary shunt [9].

The amount of work that respiratory muscles must do to maintain an optimal level of $PaCO_2$ depends on the type of ventilation. Each individual, depending on the elastic and resistance characteristics of his respiratory system, has an optimal theoretical rate where a reduction in compliance implies that it increases, whereas it is reduced when resistance increases. In clinical practice most patients with a pulmonary disorder use a ventilation mode that requires less respiratory work, and respiratory rate is often close to the optimal hypothetical one. Theoretically it is therefore possible to classify pulmonary diseases as restrictive or obstructive diseases on the basis of high or reduced respiratory rate measurements, but the respiratory centers are regulated also by cortical influences and by mechanical or irritation stimuli coming from the respiratory system that may gain the upper hand over the energy advantage.

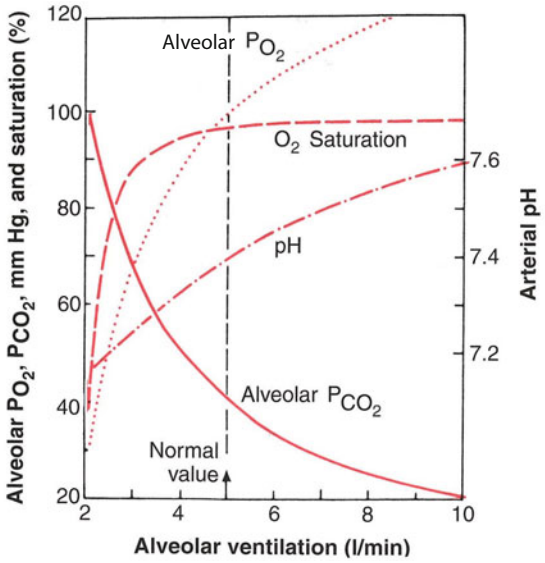

Fig. 57.6 Blood gases analysis variations related to alveolar ventilation (adult). Reproduced from [10], with permission

57.6 Diffusion

The next step after the cyclical renewal of alveolar air is the diffusion of O_2 from the alveoli to the blood, and of the CO_2 in the opposite direction [11]. O_2 and CO_2 move from high partial pressure areas to others where the partial pressure is low as a result of a process of passive physical diffusion. The diffusion of the gases across the alveolar wall is described by Fick's law:

$$\dot{V}\,\text{gas} = A \times K \times (P_1 - P_2)/S$$

This law states that the volume of gas that diffuses per unit of time ($\dot{V}$gas) is proportional to the surface area (A), to a diffusion constant (K) and to the partial pressure difference ($P_1 - P_2$), and it is inversely proportional to the thickness of the alveolar wall (S). The diffusion constant K is, in turn, proportional to the solubility of the gas and inversely pro-

portional to the square root of its molecular weight. The CO_2 has a molecular weight that is not very different from that of the O_2, but it has higher solubility, and so it diffuses about 20 times more rapidly than the O_2 through the blood-gas barrier.

At the blood-gas barrier the flow pressure for the O_2 is around 60 mmHg (100 mmHg alveolar PO_2; 40 mmHg PO_2 capillary venous blood); the O_2 crosses the membrane rapidly and the PO_2 increases just as rapidly in the blood. It must be pointed out that the blood PO_2 reaches the values corresponding to the alveolar air just before the red cells have completed half of their journey inside the lung capillaries, whose overall duration is around 0.75 sec; therefore there are huge possibilities for compensation.

Moreover, thanks to the special form of the Hb dissociation curve, a high partial pressure difference persists between alveolar gas and blood also when the Hb is near total saturation. For instance Hb is saturated at 94% with a PO_2 of 70 mmHg, when there is still a 30 mmHg gradient.

The time it takes the capillary CO_2 to equilibrate against the alveolar CO_2 is more or less the same as the time taken by the O_2 in spite of the fact that the diffusion velocity of the CO_2 is 20 times higher than that of the O_2. In this case, however, we must consider that the partial pressure gradient between capillaries and alveoli is only 5 mmHg.

57.7 Pulmonary Circulation and Ventilation/Perfusion Ratio

The fetal pulmonary arterial muscle thickness increases during the last quarter of pregnancy blocking the perfusion of the lung circulation, which is rapidly developing. Of these two phenomena, the latter is most prevalent and results in a gradual reduction in pulmonary vascular resistances. The degree of development of pulmonary circulation affects the clinical presentation of neonatal cardio-respiratory diseases where the alterations in the ventilation/perfusion ratio are determined by the capacity, or lack thereof, to increase lung vascular resistances following stimuli such as hypoxia and acidosis, with hemodynamic patterns ranging from extrapulmonary shunt to pulmonary overflow.

Let us look at the ventilation/perfusion ratio ($\dot{V}_A/\dot{Q}$) of a term newborn in physiological conditions: their cardiac index (cardiac output referred to body surface) is approximately 3.5 L/min/m^2; since body surface is around 0.25 m^2, the value of the output is 0.8–0.9 L/min. Considering a respiratory rate of around 45 breaths/min, alveolar ventilation is about 700–800 mL/min; the result is a $\dot{V}_A/\dot{Q}$ ratio of 0.8–1 as in the adult. This means that each air volume that arrives at the alveolar surface exchanges with an equal amount of capillary perfusion blood. These values are indicative, but they do give an idea of the order of magnitude of these values.

57.8 Transfer of O_2 and CO_2

In physiological conditions O_2 is transferred to the tissues almost entirely in combination with the Hb of the red cells; only 3% is dissolved in the aqueous phase of the plasma. It must be noted that 1 g of Hb may bind to a maximum of 1.34 mL of O_2 and, since the normal concentration of Hb is about 15 g/100 mL, it may bind 20 mL of O_2 or, to use the generally accepted expression, 20 volumes of O_2 [12].

The amount of O_2 that binds to Hb in the erythrocytes is directly proportional to the PO_2 in the plasma and is plotted graphically as a dissociation curve of hemoglobin (Fig. 57.7). The italics S shape of the Hb dissociation curve is due to the functional interdependence or cooperative effect of its four hemes. As a result of this effect, while the first heme combines slowly with O_2, the second, under the influence of the first that has already been assigned, will combine more rapidly, and so on. Fetal Hb has a greater affinity for O_2 compared to that of the adult; its dissociation curve is shifted to the left and has a greater saturation at the same PaO_2 (the position of the Hb curve is defined by the P_{50}, which is the PaO_2 at which the Hb is 50% saturated; the P_{50} of the HbF is 19 mmHg , that of the HbA 26 mmHg). The shift to the right of the curve, denoting a reduced affinity of the Hb for O_2, is caused by a reduction in pH; this facilitates the transfer of O_2 to the tissues (Bohr effect).

The share of Hb bounded to O_2 is expressed as percentage saturation (SaO_2%); this is equal to the O_2 content in the blood (Cont. O_2) divided by the O_2 transport capacity of the Hb (Cap. O_2), times 100:

$$SaO_2\% = \text{Cont. } O_2/\text{Cap. } O_2 \times 100$$

It must be pointed out that hemoglobin saturation expresses only a percentage and not an amount or volume of O_2.

The special form of the Hb dissociation curve in its upper part means that saturation decreases only slightly with considerable drops in PO_2; for the same reason, considerable increases in alveolar PO_2 do not cause a significant increase in O_2 content since maximum O_2 saturation cannot exceed 100%.

The steepness of the lower part of the curve shows that the peripheral tissues receive large amounts of O_2 with small decreases in capillary PO_2. In venous blood PO_2 is around 40 mmHg, which corresponds to a 75% saturation; this means that during tissue perfusion only 25% of the total content is used; in practice about 5 mL for every 100 mL of blood (Fig. 57.7).

The transport of CO_2 in the blood is not as complex as the transport of O_2 [13]. CO_2 is found in the blood as a free gas in solution (up to 10% since it is 20 times more soluble than O_2 in the plasma) or in a chemical combination with hemoglobin and plasma protein in the form of carbamin-compounds (5–10%) and above all as bicarbonate ions (70–90%). The bicarbonate is formed in the blood by the following reaction:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$$

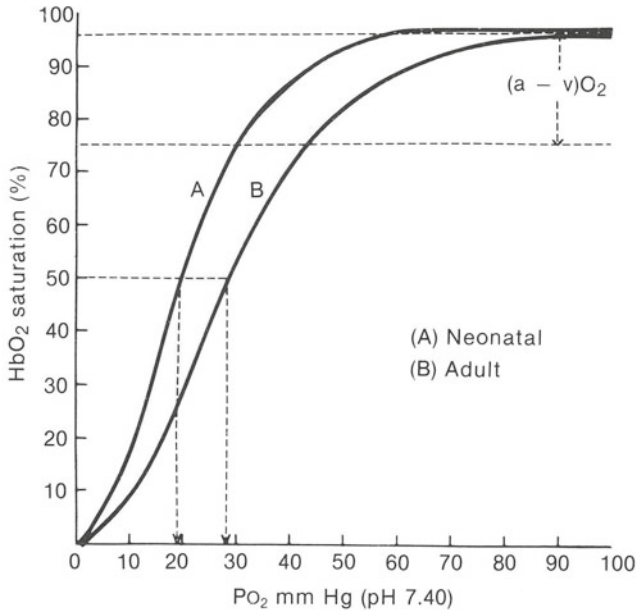

Fig. 57.7 Hb dissociation curve in a neonate (70% HbF) and in an adult in standard conditions (T= 37°C; pH 7.4). Reproduced from [4], with permission

CO_2 combines with water to form carbonic acid, which dissociates into hydrogen ions and bicarbonate ions; carbonic anhydrase, present in high concentrations in red cells, makes the reaction faster. Some of the released H^+ ions bind to the hemoglobin according to the following reaction:

$$H^+ + HbO_2 \rightarrow H^+ Hb + O_2$$

The reduced form of Hb is less acidic than the oxygenated form and it more rapidly accepts the hydrogen ions released by the dissociation reaction of carbonic acid, thus allowing a greater amount of carbon dioxide to be transported by venous blood in the form of bicarbonate ions (Haldane effect). This facilitates the uptake of CO_2 at tissue level and the inverse reaction facilitates its release into the lung capillaries where carbonic acid is converted into gas and removed from alveolar ventilation.

The acid-base status of the blood (pH) results from the ratio between bicarbonate concentration and the CO_2 in solution, and as long as this ratio is 20, the pH is stable at 7.4 [14]. (Normal arterial blood HCO_3^- concentration is 24 mmol/L, at 37°C about 0,03 CO_2 mmol for every PCO_2 mmHg in 1 L of plasma; consequently the amount of CO_2 in the blood is equal to 0,03 × PCO_2. So in normal condition the HCO_3^-/CO_2 ratio is 24/0,03 × 40 = 20.)

The blood concentration of bicarbonate is determined mainly by the kidneys and the PCO_2 by the lungs. An increase in the latter reduces the pH (respiratory acidosis) and the kidneys respond by eliminating a more acid urine, secreting H^+ ions in the tubules as $H_2PO_4^-$ or NH_4^+, while the HCO_3^- ions are reabsorbed. Kidney compensation however is hardly ever accomplished. By contrast, a reduction in PCO_2 determines an increase in pH (respiratory alkalosis) and kidney compensation occurs by eliminating a larger amount of bicarbonate. In the case of acidosis or metabolic alkalosis respiratory compensation mechanisms step in, that is hyperventilation or hypoventilation, so as to maintain the HCO_3^-/CO_2 ratio, and hence the pH, within physiological limits.

The dissociation curve of carbon dioxide shows the relationship between PCO_2 and the total content CO_2 in the blood (Fig. 57.8): at physiological levels of PCO_2 the carbon dioxide content is around 50 mL or volumes per 100 mL, with an oscillation of only 4 volumes per 100 between arterial blood and venous blood (48 mL–52 mL). In practice every 100 mL of blood that go through the lungs release 4 mL of CO_2.

In the normal range of the PCO_2 values the curve approximates a straight line without steep or flat portions; this means that an increase or slowdown in ventilation determines a proportional decrease or increase in arterial CO_2. Furthermore, the lower the PO_2, the greater the CO_2 content for a given PCO_2 (Haldane effect).

The dissociation curve of CO_2 is steeper than that of the O_2, in other words, there is greater variation in gas content for partial pressure variations. Knowing the difference between the dissociation curves of the Hb and CO_2 is essential to understand the pathophysiology of major ventilation alterations.

We have seen how 100 mL of blood transport 5 mL of O_2 from the lungs to the tissues, whereas the same amount of blood transports some 4 mL of CO_2 from the tissues to the lungs. At resting conditions, therefore, for every 100 volumes of O_2 assumed by the lungs only 80 volumes of CO_2 are eliminated, where the ratio between eliminated CO_2 and O_2 uptake is defined as respiratory quotient (RQ) and is expressed by the following formula:

$$RQ = CO_2 \text{ eliminated}/O_2 \text{ uptake} = 0.8$$

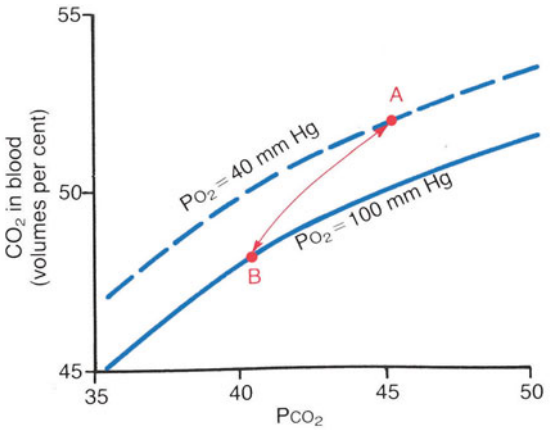

Fig. 57.8 Portions of carbon dioxide dissociation curves when PO_2 is 100 mmHg and 40 mmHg, respectively. Arrows represent the Haldane effect on the transport of carbon dioxide. Modified from [15]

57.9 Causes of Hypoxia

57.9.1 Hypoventilation

We have seen that the alveolar PO_2 and hence the arterial PO_2 is determined by the equilibrium between the speed of CO_2 removal and O_2 supply by alveolar ventilation. If the ventilation is reduced, the alveolar PO_2 drops and for the same reason the $PaCO_2$ rises, and it is the increase in $PaCO_2$ that represents a very important criterion for the diagnosis of hypoventilation because, unlike hypoxia, this is virtually the sole cause for hypercapnia. Hypoventilation is therefore characterized by an inadequate removal of CO_2 compared to its production and the relationship can be written as follows:

$$PaCO_2 = \dot{V}_{CO_2}/\dot{V}_A$$

where $\dot{V}_{CO_2}$ is the production of CO_2 and $\dot{V}_A$ is alveolar ventilation ($\dot{V}_E - \dot{V}_D$).

The gradual reduction in ventilation progressively brings the alveolar gas values closer to the venous blood values. Arterial PO_2 can be easily corrected by increasing the FiO_2, since the larger amount of O_2 that reaches the alveoli through each breath may offset the reduction in ventilation. Conversely, hypercapnia can be corrected only by increasing respiration frequency and/or the tidal volume, and may require a certain amount of time because the CO_2 is stored in large amounts in the form of bicarbonates.

The causes that determine hypoventilation are mostly non-pulmonary; amongst the most frequent causes in newborn we may mention:

- apnea spells in premature infants;
- depression of the CNS due to anesthetics or drugs;
- diseases of the CNS;
- diseases affecting the respiratory muscles (paralysis of the diaphragm, weak respiratory muscles in preterm infants, etc);
- obstruction of the upper airways (choanal atresia, Pierre-Robin syndrome, laryngomalacia, etc.);
- obstruction or mal-positioning of the tracheal tube.

Hypoventilation may also be a symptom of functional exhaustion and it may complicate pulmonary diseases when a considerable increase in work of breathing is required.

57.9.2 Abnormal Diffusion

Normal lungs present extensive compensation possibilities to ensure O_2 diffusion. It was pointed out earlier that blood and alveolar PO_2 and PCO_2 are already in equilibrium even before the blood completes half of its journey along the pulmonary capillaries. It is however possible that in some pathological conditions, like pulmonary edema or interstitial fibrosis, diffusion may be slowed down to such a rate as to make the equilibrium between PO_2 of the alveolar gas and that of the capillary blood incomplete. It is not clear, as yet, what the role of diffusion is in determining hypoxia in these disorders, but it is likely that most of the pathogenic mechanism is due to alterations in the ventilation/perfusion ratio. However hypoxia, secondary to a diffusion defect, may be rapidly redressed by administering O_2; the increase in alveolar PO_2 helps overcome the resistance to diffusion caused by changes in the membrane.

Alterations in diffusion never involve CO_2 given the rapidity with which it diffuses; in such conditions the $PaCO_2$ is often slightly decreased because of the hyperventilation caused by hypoxia.

57.9.3 Extrapulmonary Shunt

The term extrapulmonary shunt refers to the passage of blood from the right sections to the left sections of the heart without crossing the ventilated pulmonary areas. The arterial PO_2 drops when the desaturated blood mixes with the oxygenated blood.

Fig. 57.9 shows the effect of the increase in FiO_2 at various levels of shunting: when the latter exceeds 30%, the O_2 is no longer capable of redressing the hypoxemia and this characteristic makes the difference between an extrapulmonary shunt and the other causes of hypoxia. The mixing of arterial blood and shunt blood causes a considerable drop in PaO_2 because the dissociation curve of the O_2 is flat in its higher portion: it is therefore possible to show up minor shunts by measuring the arterial PO_2 during 100% O_2 inhalation (hyperoxic test). By contrast, if hypoxemia is secondary to other causes (hypoventilation, limitations to diffusion, inhomogeneities in the $\dot{V}_A/\dot{Q}$ ratio), during 100% O_2 inhalation, the

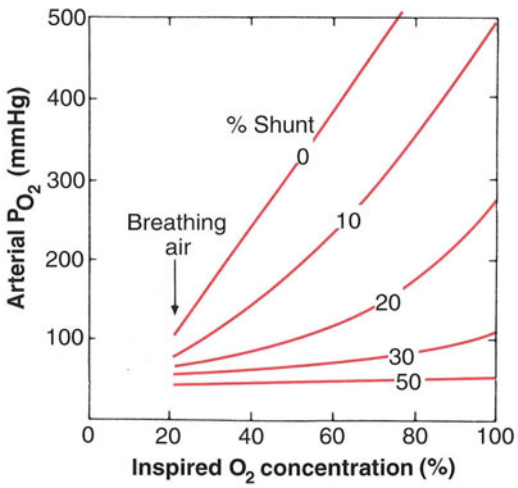

Fig. 57.9 Arterial PO_2 variation with different inhaled O_2 concentrations and different shunt percentage. Reproduced from [10], with permission

PaO_2 reaches levels that are virtually comparable to those observed in normal individuals, albeit over longer time intervals. A healthy individual who inhales 100% O_2 has an ideal PaO_2 of 673 mmHg (760 mmHg – 47 mmHg H_2O – 40 mmHg CO_2 = 673 mmHg).

The $PaCO_2$ is not usually increased in the presence of an extrapulmonary shunt because the hypoxia and hypercapnia of non-oxygenated blood cause an increase in alveolar ventilation; the latter is effective in reducing the $PaCO_2$, but totally ineffective in increasing the PaO_2. As described earlier, the reason is to be found in the shape of the two dissociation curves; the CO_2 curve is virtually straight in the physiological zone, with the result that an increase in ventilation increases the elimination of CO_2. By contrast, the shape of the Hb dissociation curve, which is flat in its upper portion, does not allow the increase in ventilation to bring about an improvement in the O_2 contained in the blood.

During neonatal age, several diseases are characterized by a right-left shunt through fetal channels for the increase of pulmonary resistances; in this case oxygen therapy is a critical element in solving pulmonary vasoconstriction and in reducing the shunt.

57.9.4 Alterations in the Ventilation/Perfusion Ratio

Alterations in the ventilation-perfusion ratio ($\dot{V}_A/\dot{Q}$) are undoubtedly responsible for most of the defects in the exchange of O_2 in pulmonary diseases and rarely for CO_2 defects [16]. The reason hypercapnia rarely occurs with alterations of the $\dot{V}_A/\dot{Q}$ ratio is because the high degree of efficiency of the chemoreceptors that keeps the arterial CO_2 constant by increasing ventilation. As shown earlier, the linear shape of the CO_2 dissociation curve ensures this compensation.

Also in the healthy lung there are regional variations in the $\dot{V}_A/\dot{Q}$ ratio, but the overall effect on the total gas exchange is negligible. The distribution of ventilation in particular is regulated by tissue compliance and by the resistance of the airways; differences in these factors in the pulmonary district cause a non-uniform distribution of ventilation.

Let us now look at the consequences of the two extreme variations of the $\dot{V}_A/\dot{Q}$ ratio, bearing in mind that in practice intermediate degrees of these alterations coexist:

- $\dot{V}_A/\dot{Q} = 0$. In practice the alveoli are not ventilated but perfused (shunt effect). In this case there is no gas exchange between blood and air, but an equilibrium sets in between venous blood and the air in the non-ventilated alveoli; inside the latter the partial pressures will be the same as those of venous blood (Fig. 57.10b). In this way an intrapulmonary shunt is caused because the blood that perfuses the non-ventilated zones is not oxygenated, nor is the CO_2 removed; the consequences are identical to those caused by an extrapulmonary shunt.
  In this case an efficient compensation mechanism intervenes, hypoxic vasoconstriction, which involves the capillaries that perfuse the areas where ventilation is reduced or totally absent; the local blood flow is diverted to the ventilated alveoli and the deleterious effects on the exchange of gases is considerably reduced. It is interesting to point out that when this occlusion of the airways persists, the alveoli end up collapsing because of the diffusion of gas in the blood, and the formation of atelectasis due to reabsorption is more rapid if the patient breathes O_2 at high concentrations. Indeed, nitrogen has a low degree of solubility and its presence provides support for the alveoli and delays their collapse in the case of obstruction of the airways. Oxygen dependence, which is typical of children with bronchopulmonary dysplasia (BPD), is almost certainly due to the presence of multiple shunts having a low $\dot{V}_A/\dot{Q}$ ratio.

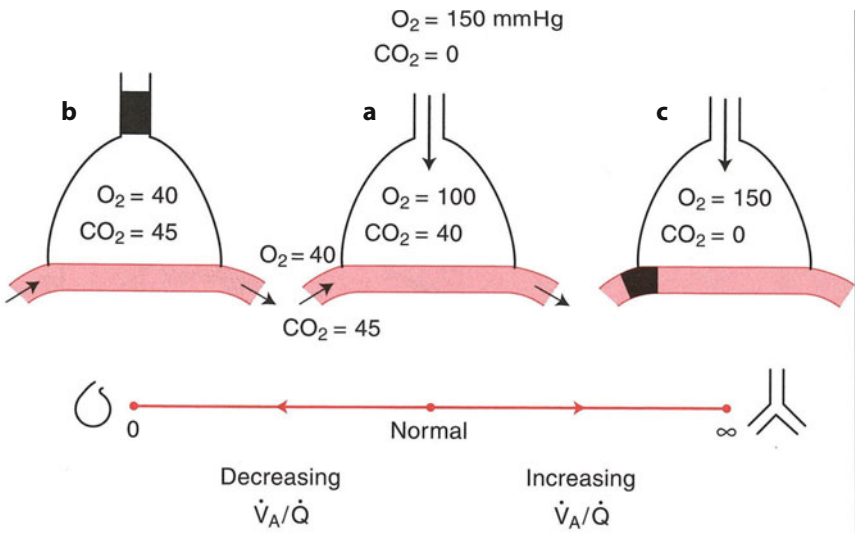

Fig. 57.10 Effects of altering the ventilation-perfusion ratio on the PO_2 and PCO_2 in a lung unit. Reproduced from [17], with permission

- $\dot{V}_A/\dot{Q}$ = infinite. In practice the alveoli are ventilated but not perfused (dead space effect). Also in these conditions there is no blood-air exchange; and indeed, instead of having partial pressures similar to those of venous blood, the air in the alveoli has the same partial pressures as atmospheric air. In other words, O_2 is not transferred to the blood and CO_2 is not removed. This quota of ventilation remains unused (physiological dead space) and increases the respiratory dead space thus causing a reduction in alveolar ventilation (Fig. 57.10c). It is not possible to describe the gas composition of the blood coming from these units because perfusion is totally absent, but clearly the PO_2 and the PCO_2 of the blood will always be closer to those of inspired air as the perfusion is progressively reduced. The units with a high $\dot{V}_A/\dot{Q}$ ratio add little O_2 to the blood compared to the decrease caused by the alveoli with a low $\dot{V}_A/\dot{Q}$ ratio, and the reason is always to be sought in the shape of the Hb dissociation curve.

57.9.5 Hypoxia Due to Anemia and Hypoperfusion

It is worth remembering that to ensure an adequate O_2 supply to the tissues, a normal Hb concentration is required and also the cardiac output must be adequate. The efficacy of the latter may be determined indirectly by assessing skin color and skin temperature, peripheral pulses, gas-analysis values, diuresis and directly by means of color-Doppler echocardiography. And finally, it must be pointed out that a reduction in Hb causes a decrease in the O_2 content without any variation in the PaO_2; an anemic patient may therefore have a good PaO_2, but a low O_2 content.

57.10 Conclusions

After having schematically described the pathophysiology of the possible causes of hypoxia it is worthwhile underlining that in practice these mechanisms often coexist and contribute to determining respiratory failure.

The most typical example in neonatology is RDS, where the lack of surfactant and an alteration in pulmonary compliance cause the formation of atelectasis and the uneven distribution of ventilation; as a consequence the $\dot{V}_A/\dot{Q}$ ratio is altered. The edema and the presence of exudates at the alveoli may have a negative impact also on the diffusion of O_2. The resulting hypoxia, combined with acidosis, further worsens the ratio due to the increase in vascular resistance with the formation of shunts in the fetal channels and hypoperfusion of the pulmonary circulation. Finally, owing to the considerable increase in respiratory work, the patient may present with hypoventilation symptoms after an initial phase characterized by tachypnea.

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58

Control of Breathing in Newborns

Ruben Alvaro and Henrique Rigatto

58.1 Introduction

There are at least three important considerations regarding the study of the control of breathing during the neonatal period. First, the neonates are noncooperative subjects. This means that we must study their respiratory control without their being aware and try to compare the measurements with those of the adult under similar conditions. This is difficult to do. Second, measurements in the neonate are usually made, by necessity, in the decubitus position, whereas those in the adult subject are usually made in the sitting or standing position [1, 2]. Third, babies are usually studied with a nosepiece because they are nose breathers; adults are usually studied using a mouthpiece. These methodological differences have made comparison of breathing in newborns with that in adult subjects difficult to interpret. There is currently a major need for studies to be done using similar methodology. Unless there is some consistency in the methodology, it is hard to define what is actually distinct or unique about the control of breathing in the neonate. In recent years, we have experienced tremendous advances in the field of respiratory control, and we are now witnessing the initial discovery of several of the genes that control the development and maturation of multiple neurally controlled respiratory functions

In this chapter we review some of the concepts and the progress made in the area of control of breathing in the newborn. We also highlight major developments and critically analyze the scientific foundations of our knowledge in this area.

58.2 Breathing Pattern at Rest

The neonate, and particularly the premature infant, breathes irregularly. There is significant breath-to-breath variability and long stretches of periodic breathing in which breathing and apnea alternate [2–4]. Haldane's statement that "the surprising fact is not that we breathe regularly, but that we do not breathe periodically most of the time" applies more at this age than at any other [5]. The resting breathing pattern of the neonate is not sleep-state dependent, although sleep greatly modulates it [6, 7].

Sleep has traditionally been divided into quiet, REM, transitional, and indeterminate states. Twenty-nine percent (29%) of sleep time in neonates is spent in quiet sleep, 33% in REM, 7% in transitional sleep, and 31% in indeterminate sleep. The proportion of quiet sleep increases with age, while the amount of REM decreases with age. The proportion of wakefulness decreases with decreasing gestational age and in very immature infants, it becomes difficult to define wakefulness or arousal [8, 9]. We have shown that periodic breathing, a common breathing pattern in premature infants in which they alternate between breathing intervals and apneas lasting 5–10 seconds, occurs in the three states, wakefulness, REM, and quiet, but its prevalence is increased in REM sleep [6]. It is frequently stated in textbooks that in quiet sleep, in analogy with criteria used for adult subjects, breathing is regular. However, we and Prechtl have clearly shown that periodic breathing is common in quiet sleep [2, 10, 11]. The difference is that periodic breathing in quiet sleep is regular, that is, the breathing and apneic intervals are of similar duration, and very irregular in REM sleep. The most well-defined periodic breathing observable in small babies is in quiet sleep during tracé alternant. Therefore, there are two major differences between neonates and adults regarding staging of sleep state. One difference relates to the patterns of breathing observed in quiet and REM sleep states and the other to the presence of the trace alternant electroencephalogram (EEG) during quiet sleep in the neonate. As this pattern subsides after 44 weeks' postconceptional age, it is not used in adults to characterize quiet sleep. Finally, the overall minute ventilation is increased in REM sleep as compared with quiet sleep, and this is due to a primary increase in respiratory frequency with little change in tidal volume [1, 2, 6].

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

58.3 Periodic Breathing and Apnea

Periodic breathing, defined as pauses in respiratory movements that last for up to 20 seconds alternating with breathing, is common in preterm infants. When the respiratory pause is longer than 20 seconds, it is called apnea [12]. Although the duration used to distinguish periodic breathing and apnea is arbitrary, it has proved useful and has been widely adopted. Periodic breathing is not as harmful as apnea since the respiratory pause is short and the decrease in heart rate is minor. In contrast, apnea is a more serious condition, the respiratory pause being longer and frequently associated with decreases in heart rate below 80 beats/minute [12, 13]. In very small preterm infants, significant bradycardia and desaturation can occur with very short apneic pauses, likely related to their higher oxygen consumption per unit weight and their relatively smaller lung volumes and oxygen stores than the term infants and adult subjects. In this instance, therefore, the length of the respiratory pause is not a very useful indicator of severity of the disruption in breathing. For this reason, many centers, including ours, have decided to rely on heart rate and oxygen saturation as the primary indicators of severity.

Apneic episodes in the neonate are classified according to the absence or presence of breathing efforts during the period of no airflow [14]. Central apneas are those with no flow and no observable breathing efforts. Obstructive apneas are those with no flow despite breathing efforts. Mixed apneas begin as central and end as obstructive apnea. Breath-holding apneas are those in which flow stops at mid-expiration, and the remaining expiration occurs just before breathing starts again. A new method of classifying apneas, based on a magnified cardiac-induced pulse observed on the respiratory flow tracing, has been recently described by us. This method is able to detect presence and timing of airway obstructions with great precision. Using this method it is obvious that some apneas, previously classified as central because of absence of respiratory efforts, are indeed obstructive (silent obstruction) (Fig. 58.1) [15, 16]. We have been using this method extensively in our experimental studies, but its use in routine clinical care is not yet established.

In preterm infants with underlying disease followed longitudinally over a period of 3 months, central apneas predominated and purely obstructive apneas were rare [14]. Obstructive apneas are more commonly noted as part of a mixed apneic event. Most short apneas are central, whereas long apneas are mixed. The mechanism of obstruction in mixed apneas is unknown and it is not completely clear whether the obstructed respiratory efforts prolong the apnea or whether a longer initial central pause predisposes the airway to collapse at the end of the apnea. We found that airway closure, as measured by the absence of cardiac oscillations on the respiratory flow tracing, was present in about 20% of central apneas and preceded 59% of the obstructed respiratory efforts in mixed apneas. This airway closure during central apneas did not limit the severity of oxygen desaturation or the decrease in heart rate. We also found that although the length of mixed apneas was longer than central apneas, the length of the initial central component of the mixed apneas was significantly shorter than the length of central apneas, suggesting that respiratory efforts against a closed airway prolong apnea. These respiratory efforts against a closed airway also aggravated the oxygen desaturation and the decrease in heart rate that are usually associated with apneas [16]. In preterm infants recovering from respiratory support, with some degree of residual lung disease (bronchopulmonary

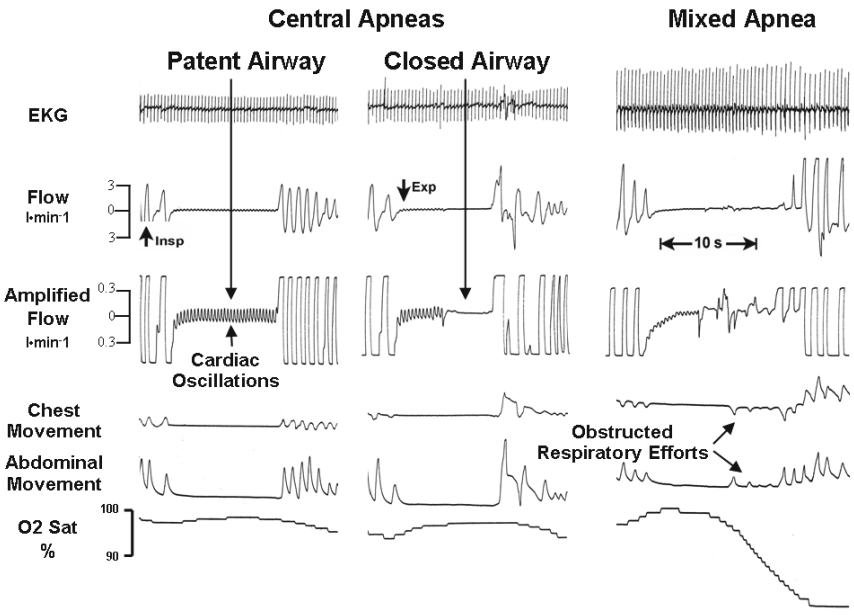

Fig. 58.1 Representative tracing: Examples of three types of apneas diagnosed by absent (central apneas) or present obstructed respiratory efforts (mixed apnea). Central apneas are further classified using the amplified cardiac airflow oscillation into those with open airway (oscillations present) and those with closed airway (oscillations absent). Cardiac oscillations and respiratory effort signals are indicated. The flow channel shows baseline respiratory flow with minor amplification. The amplified flow represents a 10-fold amplification of the flow signal by the chart recorder so that cardiac oscillation may be easily analyzed [16]

dysplasia), the prevalence of obstructive apneas appears to be increased, comprising up to 48% of the apneas in some studies. There is no clear explanation for the obstruction, but it seems to be at the level of the larynx [17].

Periodic breathing and apnea are clearly a consequence of a disturbance of the respiratory control system, but the precise mechanisms are unclear. Investigators in this area tend to believe that the negative feedback loop controlling respiration is affected by multiple factors related primarily to anatomic and physiologic immaturity that affect many levels of the respiratory control system including central and peripheral chemoreceptors. For example, arborization of dendrites at 30 weeks' gestation is meager, and neuroconduction and synaptic relay are impaired [18]. Delays in traffic of neuromessages may then make the system oscillate. Unfortunately, we do not know how much immaturity is needed for a given impairment in neurophysiologic traffic. Oscillation in arterial gas tensions, changes in circulation time, incoordination of the respiratory pump owing to a compliant chest wall, and changes in sleep state may all contribute to this instability of the respiratory control system [12]. Many inhibitory neurotransmitters and neuromodulators, including adenosine, prostaglandins, endorphins and GABA have been also implicated in the pathogenesis of periodic breathing and apnea [19].

There has been controversy in the literature on whether periodic breathing and apnea are mechanistically different or whether long apneas are just a step further in the basic respiratory disturbance that induces the short apneas of periodic breathing. In a study carried out in our laboratory we were able to show that i) a prolonged apnea almost never occurred in the absence of preceding short apneas, and ii) the risk of a prolonged apnea occurring increased significantly when the preceding period contained an increased number of apneic episodes, increased duration of the longest apneic interval, or increased duration of the apneic time [20]. More recently we have shown that the periodic breathing cycles in REM but not in quiet sleep were associated with progressive decrease in minute ventilation and oxygenation likely related to the mechanical and chemoreceptor limitations known to be present in this sleep state. We believe that periodic breathing, especially during REM sleep, is a marker for apnea since apneas almost never occur abruptly in infants breathing regularly, but only in infants whose respiratory pattern is characterized by significant periodicity.

Compared with infants who breathe continuously, neonates breathing periodically have lower arterial PO_2 values and their peripheral chemoreceptors are more hyperactive as reflected by the longer apneic period and more pronounced immediate decrease in ventilation in response to inhalation of high oxygen mixtures [13, 21, 22]. Thus, the basic reason for background instability appears to be the major contribution of the peripheral chemoreceptor drive to normal breathing at this age. Indeed, the arterial PO_2 tension of these infants sits on the steep portion of the minute ventilation-arterial PO_2 regression curve for human adults. This means that small changes in baseline arterial PO_2 produce large changes in baseline ventilation. Hypoxia may be a contributing factor, because inhalation of a low-oxygen mixture easily induces periodic breathing and apnea in these infants.

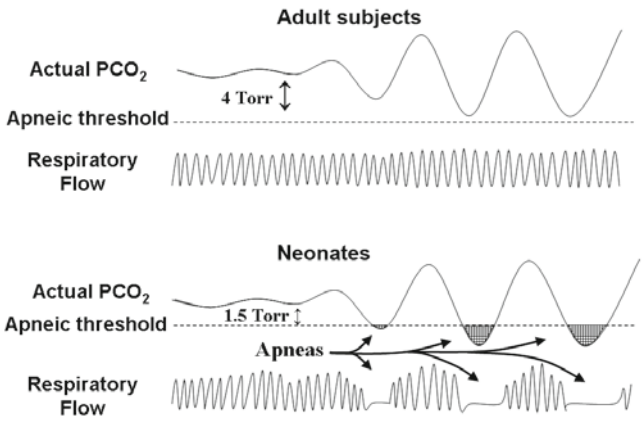

Fig. 58.2 Diagrammatic representation of the relationship between the CO_2 apneic threshold and the baseline or actual PCO_2 levels in neonates and adults. Because of the proximity of these two levels in neonates, PCO_2 is much more likely to dive below the apneic threshold than in the adult

We found that the average CO_2 apneic threshold in preterm infants is only 1.5 Torr lower than the eupneic PCO_2, whereas in adults the average is ~3.5 Torr lower [23]. The closeness of eupneic and threshold $PACO_2$ likely confers a great vulnerability to respiratory stability in these infants. It is not surprising, therefore, that brief startles, movements, or change in sleep state could allow eupneic PCO_2 to dive below the PCO_2 apneic threshold, inducing periodic breathing and apnea in these infants (Fig. 58.2). We believe that the key element responsible for this narrow difference is the well-known hypoxemic status of these infants. Xie and colleagues have recently shown in adults that the time course of the occurrence of apnea after transient hyperapnea was consistent with a peripheral chemoreceptor mechanism. In this study, hypoxia shortened the apnea latency and narrowed the eupneic-apneic $PACO_2$ threshold while hyperoxia delayed the onset of apnea and widened the eupneic-apneic $PACO_2$ threshold [24]. The same group had previously shown that the smaller difference between eupneic and apneic $PACO_2$ during hypoxia was due to a disproportionate reduction in the eupneic $PACO_2$ rather than a higher apneic threshold [25]. The low arterial PO_2 in newborn infants may keep the eupneic PCO_2 relatively low, not by hyperventilation as in adult subjects but by a decrease in metabolism, which parallels the decreased in arterial PO_2.

The sleep state appears also to be a contributing factor, since periodic breathing and apnea are more frequent in REM sleep than in quiet sleep. The neonate sleeps almost uninterruptedly, continuously alternating between REM sleep and quiet sleep. This pattern increases instability in the respiratory control system. Indeed, minor alterations during sleep, such as a startle or a sigh, produce apnea in these infants. The almost

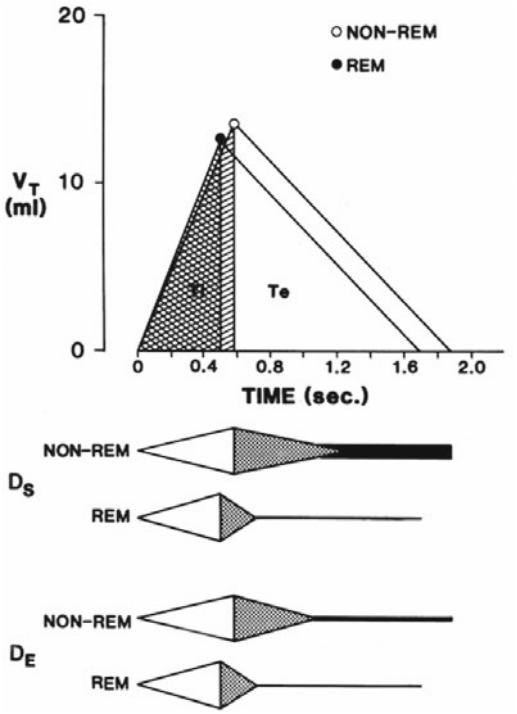

Fig. 58.3 Diagrammatic changes in tidal volume, timing, and diaphragmatic EMG in non–rapid eye movement (REM) (quiet) and REM (active) sleep. Note that total phasic activity diminishes from non-REM to REM sleep. Also, in both sleep states, it is shorter in esophageal (DE) than in surface EMG (DS). Expiratory phase activity as a proportion of total phasic activity decreases significantly from non-REM to REM sleep. From [26]

continuous change in baseline ventilation during sleep is what Haldane called "the hunting of the respiratory centre" [24].

Although sleep modulates breathing, it does not cause apnea; apnea also occurs during wakefulness. The high prevalence of apnea during REM sleep may be related to muscle activity in this stage. During REM sleep, the tone of the intercostal muscles is abolished in conjunction with a decrease in diaphragmatic activity and in the tone of the adductor muscles of the upper airway, a combination of factors likely to induce chest distortion, impairment of the braking mechanism during expiration, pulmonary collapse, and apnea (Fig. 58.3) [12, 26]. When chest distortion occurs, diaphragmatic work increases by about 40%, adding to the mechanical impairment. This observation is compatible with the finding that the application of continuous negative pressure around the chest tends to abolish apnea [27].

58.4 Chemical Regulation

Inhalation of carbon dioxide increases ventilation during REM and quiet sleep in newborn infants. The response to steady-state inhalation of carbon dioxide is the same in these two sleep states, but the response during rebreathing of carbon dioxide is less in REM than in quiet sleep [1, 6, 10, 28] (Fig. 58.4). We postulated that the differences in response with these two techniques relate to the fact that using rebreathing, it is possible to measure the response in "phasic"

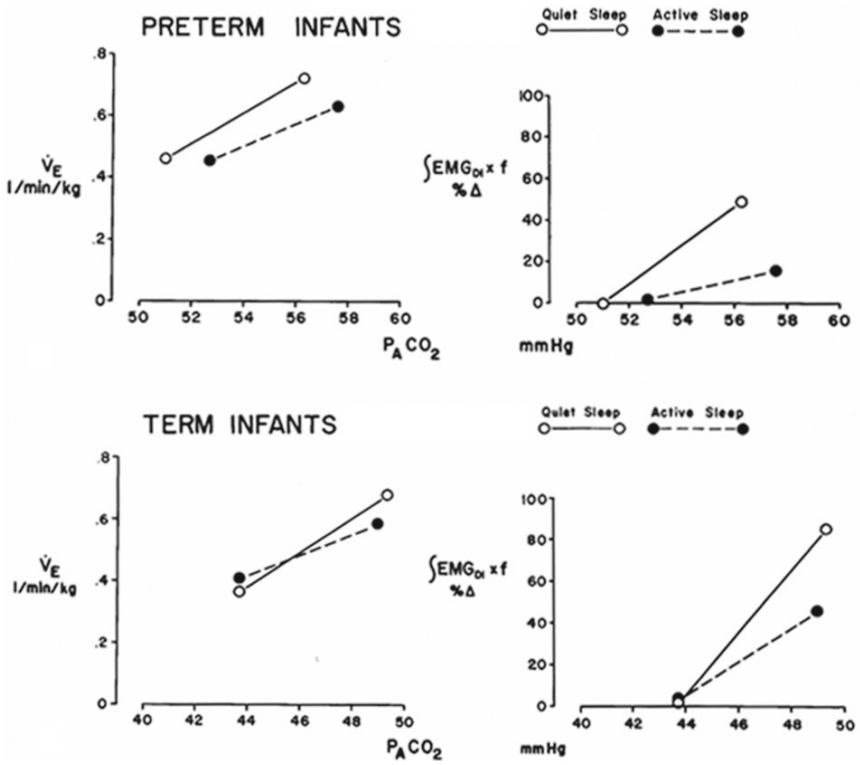

Fig. 58.4 The ventilatory response to CO_2 rebreathing in neonates. Note that (**a**) preterm and (**b**) term infants showed a decreased response to CO_2 in phasic rapid eye movement sleep as compared with quiet sleep [10]

REM only, whereas using the steady-state technique the response always covers both "phasic" and "tonic" REM sleep [10]. As the carbon dioxide response in "tonic" REM is the same as that during quiet sleep, the results using the steady-state method tend to resemble those in quiet sleep [29, 30].

The pattern of breathing observed with inhalation of carbon dioxide varies with the percentage of carbon dioxide inhaled. If the percentage of inhaled carbon dioxide is low (<2%) during steady-state inhalation, the response consists primarily of an increase in tidal volume [2]. If the percentage of inhaled carbon dioxide is high (>2%), the response in both sleep states consists of an increase in respiratory frequency and in tidal volume [10, 21]. Periodic breathing is abolished with a small increase in inhaled carbon dioxide of about 1–2% [2, 21]. This response has been attributed to the increased central drive and increased stores of carbon dioxide, with better buffering capacity for the oscillations in $PaCO_2$.

Inhalation of low oxygen produces an immediate increase in ventilation (1 minute) followed by a later decrease (5 minutes) [4, 31–33]. The response is similar in wakefulness, REM, and quiet sleep, although hyperventilation seems slightly more sustained during late hypoxia in quiet sleep [1]. The more sustained hyperventilation in these infants during quiet sleep reflects the more autonomic control during this sleep state, the system being more responsive to chemical stimuli [29, 30]. The immediate increase in ventilation reflects peripheral chemoreceptor stimulation and is associated with an increase in frequency and in tidal volume. The late response is primarily manifested by a decrease in frequency [13, 31]. The mechanism responsible for this response is still unclear, and may vary according to species. In humans it is likely related to central release of inhibitory neuromodulators [34]. This late hypoxic ventilatory depression is likely mediated through several complex mechanisms. In addition to hypoxic hypometabolism [35], several neuromodulators play a role in the hypoxic ventilatory depression including adenosine [36], γ-aminobutyric acid (GABA) [37], serotonin (5-HT) [38], opioids [39], and platelet-derived growth factor (PDGF-β) receptors [40]. However, experiments in kittens and in newborn monkeys suggest that the late decrease in ventilation may be a mechanical effect rather than a depression of the central respiratory neurons [41, 42]. In these experiments, diaphragmatic activity and frequency remained elevated during hypoxia, but tidal volume decreased below control values during late hypoxia. These experiments were carried out during quiet sleep. The peculiar response of the neonate to low inhaled oxygen is of great clinical significance. Infants who are borderline hypoxic tend to breathe periodically or develop apneic spells. Hypoxia can induce periodic breathing in these infants, as shown previously [43]. The relief of these apneic spells, which are frequently associated with bradycardias, can be obtained by increasing the inspired oxygen concentration [13, 21].

Administration of high concentrations of oxygen, on the other hand, produces an immediate decrease in ventilation followed by hyperventilation, a response that is similar during wakefulness, REM, and quiet sleep. These findings suggest a lack of major differences in the activity of the peripheral chemoreceptors during these sleep states [12, 44]. The immediate decrease in ventilation following the administration of 100% oxygen is related to a decrease in frequency (apnea being common in preterm infants) and a decrease in tidal volume. The late increase in ventilation with oxygen is likely related to cerebral vasoconstriction with increased H^+ concentration at the chemoreceptor site [45].

58.5 Upper Airways and Pulmonary Reflexes

The laryngeal chemoreflexes (LCR) comprise a group of reflexes triggered by the contact between liquids and receptors of the laryngeal mucosa. The receptors involved appear to be unmyelinated nerve fibers located immediately beneath the laryngeal mucosal epithelium [46]. Stimulation of the laryngeal mucosa, either chemically or mechanically, causes laryngospasm, swallowing, central or mixed apnea, oxygen desaturation and bradycardia [47]. This reflex-induced apnea is mediated through superior laryngeal nerve afferents [48]. Immature LCR, characterized by an exaggerated inhibitory cardio-respiratory response, are mainly observed in preterm infants or in conditions where upper airway inflammation is present [49]. Some authors have suggested that up to 70% of apnea of prematurity as well as the apneas associated with respiratory sincitial viral infection are related to LCR reflexes [49–51]. With maturation, the duration and severity of apneas secondary to LCR reflexes decrease. This is in part related to decrease stimulation of the LCR due to improved coordination of upper-airway muscles and to the increase in the excitatory respiratory related neurons within the respiratory network that counterbalance the inhibitory afferent input from the LCR [52, 53].

The inflation reflex of Hering-Breuer is much more active in the newborn period than in adult life [54, 55]. Small increases in lung volume cause apnea. This response is so powerful in the newborn that many have used this inflation to produce apnea and then study the mechanical properties of the respiratory system during the passive expiratory phase following apnea. The action of the stretch receptors is much influenced by sleep, being abolished during REM sleep. The irritant receptors are also poorly developed in preterm infants, and the mediated reflexes are also abolished during REM sleep [56]. Therefore, airway mechanisms responsible for clearing, such as cough, are impaired during REM sleep. The paradoxical reflex of Head is commonly observed in the neonate in the form of a sigh [57]. Many attribute the high prevalence of sighs to the greater need for lung recruitment at this age [58]. Sighs are more frequent in REM than in quiet sleep and are also more frequent during periodic than regular breathing. During periodic breathing, a sigh usually appears during the first or second breath after apnea. When it occurs

during regular breathing, it tends to be followed by short apneas [28]. Efforts to discover the mechanisms triggering sighs have been fruitless. Thach and Tauesch showed that asphyxia does not seem to be a stimulus [58]. Alvarez and colleagues, however, have observed that airway occlusion in the presence of hypoxia predisposes to sighs [59].

When studying the morphology of sighs in infants and adult subjects we have found that the sighs in infants are relatively larger than those in adults and that while post-sigh ventilation is usually increased in adults, it is decreased in infants. Since the drive to breathe early in life is dependent on increased peripheral chemoreceptor activity, it is conceivable that the sudden increase in arterial PO_2 with sighs, could produce a rapid decline in carotid body afferent discharge leading to hypoventilation and apnea. The other almost instantaneous change that occurs with a sigh includes a decrease in PCO_2. Since the CO_2 apneic threshold is much closer to the baseline CO_2 in neonates compared with adults, the decline in CO_2 below the threshold during a sigh, could trigger an apnea or initiate an epoch of periodic breathing in infants with an immature respiratory feedback loop [23, 60]. These findings suggest that although the ability to sigh may be an important mechanism to restore lung volume, sighs have the potential to destabilize breathing and cause hypoventilation and apnea in infants at risk for inadequate control of breathing [61].

58.6 Respiratory Muscles

The activity of the respiratory muscles is much altered by sleep state. Tonic activity of most respiratory muscles is abolished during REM sleep [62–64]. The disappearance of tone in the intercostal muscles has been suggested as a major factor responsible for the increased chest distortion seen during REM sleep in infants with this condition. Lack of tone leads to chest wall collapse during inspiration, and caudal displacement of the diaphragm has to be twice as long to produce the same lung volume displacement [61]. Because of chest wall collapse, functional residual capacity is decreased in these infants during REM sleep [61]. We have found that distorted and nondistorted breaths produce the same instantaneous ventilation as long as they are of the same duration, although the work of the diaphragm is 40% greater when distortion is present.

It has been shown that in newborns, in contrast to adults, expiration is actively terminated at substantial flow rates. The role of this expiratory braking mechanism is to maintain lung volume above FRC. The newborn uses two mechanisms to actively slow expiration. The first one is the postinspiratory activity of the diaphragm [65]. This activity controls, in part, the duration of expiratory time and is also affected by sleep [66, 67]. In neonates, this activity is more pronounced in the lateral than in the crural part of the diaphragm, longer in quiet than in REM sleep, and more prolonged in preterm than in term infants [68]. The length and variability of this activity in preterm infants suggest that because of their highly compliant chest wall, these infants use the postinspiratory diaphragmatic activity as a braking mechanism whose role in maintaining lung volume and controlling expiratory time is much more important than in older children and adult subjects. The second mechanism that the newborn uses to slow expiration is the laryngeal narrowing during expiration [69]. Sleep state profoundly affects the muscular control of upper airway resistance. Studies in fetal and neonatal lambs suggest that the abductor muscles of the larynx—the posterior cricoarytenoid and cricothyroid—have inspiratory activities in parallel with that of the diaphragm, during both quiet and REM sleep. On the other hand, the adductor muscles of the larynx—the thyroarytenoid, lateral cricoarytenoid, and intraarytenoid—have a phasic expiratory activity during quiet sleep. This activity is lost during REM sleep in the fetus and in the newborn lamb [62, 63]. A reduction in adductor activity of the larynx, in conjunction with decreased intercostal and decreased postinspiratory diaphragmatic activity during REM sleep, may cause the decrease in lung volume observed during this sleep state.

58.7 Conclusions

In summary, although the basic mechanisms involved in the control of breathing during neonatal life are similar to those investigated more extensively in adult subjects, there are some unique aspects of this control primarily affecting the preterm infant. First, sleep seems to have a very profound effect during this period of life. REM sleep increases the incidence of periodic breathing and apnea, decreases ventilatory response to CO_2, enhances the late decrease in ventilation with hypoxia, increases chest distortion leading to muscle fatigue, inhibits pulmonary reflexes, and decreases upper airway tone and postinspiratory activity of the diaphragm leading to decrease lung volume during expiration. Second, the highly compliant chest wall, due to lack of mineralization, puts these newborn infants at a mechanical disadvantage which is worsened by the inhibitory effects of REM sleep. These shortcomings favor instability of the respiratory control system. Third, the low FRC of small infants means low O_2 stores with less buffering capacity and increased risk of atelectasis, leading to intrapulmonary right-to-left shunts and reduced arterial O_2 tension. This relatively hypoxic environment increases the activity of the peripheral chemoreptors and narrows the difference between baseline PCO_2 and CO_2 apneic threshold levels favoring the establishment of periodic breathing and apneas. Thus, neonates have some unique features of their respiratory control system, which may at times compromise their health. It is the understanding of these features and their intimate physiologic mechanisms by physicians and other health care workers that represents the best promise for a good quality of medical care at the time of birth.

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59

Meconium Aspiration Syndrome

Simone Pratesi and Carlo Dani

59.1 Introduction

The term meconium is derived from the Greek word *mekoni*, which means poppy juice or opium due to its tarry appearance or to Aristotle's belief that it induced sleep in the fetus. Meconium constitutes the first stool of a newborn infant. The passage of meconium typically occurs within 48 hours after birth, however it can occur in utero.

The optimal care of an infant born through meconium stained amniotic fluid (MSAF) should involve obstetrician and pediatrician together. Indeed, management of a baby at risk of meconium aspiration syndrome (MAS) starts in the delivery room, or even earlier during intrauterine life. MAS is defined as respiratory distress in an infant born through MSAF whose symptoms cannot be otherwise explained; approximately 13% of liveborn infants are born through MSAF, while MAS occurs only in 2–6% of these neonates [1]. A retrospective review of the outcomes and treatment patterns in term neonates admitted for intensive care during a ten-year period indicates an increasing likelihood of MAS with advancing gestational age (from 1.1% at 37 week gestation to 24% at >42 weeks gestation), a quite constant mortality rate of about 1.2% despite changes in care during the last decade, and a higher mortality risk for infants born smaller, outborn, delivered by C-section, who had lower Apgar scores and pulmonary hypertension [2].

Meconium is first noted to be present at 12 wk gestation in the human fetus. It is the by-product of fetal amniotic fluid, lanugo, skin cells, and vernix caseosa swallowing, it also contains cells derived from the gastrointestinal tract. Meconium composition also includes four different biliary acids (cholic, chenodeoxycholic, deoxycholic, and lithocholic) and minerals of which copper, zinc, magnesium, calcium iron, and phosphorus are the most common. In addition, it contains plasmatic proteins such as α1-antitripsin and phospholipase A_2, free fatty acids, bilirubin and enzymes. The passage of meconium from fetus into the amnion at an early stage of gestation is prevented by the lack of intestinal peristalsis due to low motilin levels, tonic contraction of the anal sphincter and a terminal cap of viscous meconium. MSAF in advanced gestation may therefore be a natural phenomenon that reflects a postterm fetus with a mature gastrointestinal tract in which motilin levels have risen. On the other hand, MSAF has been associated with perinatal conditions (such as intrauterine growth restriction, pre-eclampsia, eclampsia, maternal diabetes), which can compromise the uteroplacental circulation with resultant acute or chronic fetal hypoxia and acidosis, in the presence or absence of fetal distress, producing relaxation of the anal sphincter that occurs more easily in term than preterm neonates.

59.2 Pathogenesis

The traditional belief was that meconium aspiration occurs immediately after birth. Several investigators have suggested, however, that most cases of meconium aspiration occur in utero when fetal gasping is initiated before delivery. Thus, it is believed that MAS will sometimes occur despite appropriate airway management at delivery. There is currently no way to distinguish between the infant who has developed MAS by intrauterine respiration or gasping and the infant who has developed MAS by inhalation of meconium at the first breaths after delivery.

But is MAS really caused by a direct injury of meconium on the lung? It is unclear why some infants born through MSAF develop an aspiration syndrome whereas others do not. It is known that meconium presence under the vocal cords is associated with higher risk of MAS, but its absence does not mean the infant will not develop severe respiratory failure. According to Ghidini and Spong there are no correlations between the presence of meconium in the trachea and

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clinical symptoms, as 2/3 of babies with meconium in the trachea do not develop any respiratory syndrome and a half of those with a respiratory syndrome did not have meconium in the lungs [3]. Thus the correlation, so far considered obvious, between the presence of meconium into the airways and respiratory disease, would be not so obvious. It is suggested that other pathological events such as intrauterine asphyxia could cause the syndrome, while the presence of meconium into the amniotic fluid and the lungs only being an incidental finding or a consequence of the stress insult to the fetus. Intrauterine asphyxia would lead to a remodeling of the pulmonary vascular tree determining a thickening of the pulmonary vascular media and an abnormal vascular reactivity responsible for the persistent pulmonary hypertension associated with persistent hypoxemia frequently seen in newborn with severe MAS [4]. Autopsy data from cases of MAS severe enough to result in neonatal demise within 48 hours of birth show that muscularization of the distal pulmonary arterioles is present in close to 100% of cases. As de novo muscularization requires a minimum of 3–8 days to develop, it may actually precede the aspiration of meconium [5]. Increasing evidence suggests that a chronic in utero insult may be responsible for most cases of severe MAS as opposed to an acute peripartum event. Chronic fetal hypoxia and acidosis may lead to fetal gasping and the subsequent in utero aspiration of meconium. This is supported by autopsy findings of meconium in the alveoli of stillbirths or of infants who died shortly after birth and never developed spontaneous respirations. In contrast to these severe cases, the vigorous infant who aspirates meconium-stained fluid from the nasopharynx at birth usually develops mild to moderate disease. The pathogenic events leading to mild MAS and severe MAS seem to be different, and events antedating labor (such as chronic asphyxia or infection) are predominantly responsible for the lung disease among infants with severe MAS. This is a crucial point as it seems that the only possible prevention of severe MAS could be during intrauterine life. Undoubtedly meconium in the lungs alters the pulmonary physiology but it is not clear how much of the lung injury seen in MAS (inflammation, surfactant dysfunction, vasoconstriction, airway hyperreactivity) is ascribed to a direct toxicity of meconium on lung tissues or partially to other chronic or acute insults begininning before delivery, or even how those injuries could be partially a consequence of the start of therapy (oxygen, ventilatory support, etc.).

Mechanisms of injury in MAS are: (1) meconium mechanical obstruction of airways of variable degree, leading to zones of atelectasis with ventilation/perfusion mismatch or zones of air-trapping at high risk for air leaks; (2) pneumonitis, with an inflammatory response characterized by the presence of an elevated cell count and pro-inflammatory cytokines (IL8, IL6, Il1β, TNFα), that can adversely affect tissues and cause an increase in microvascular permeability leading to hemorrhagic pulmonary edema and exudation of plasma proteins into the alveolar space leading to (3) inactivation of pulmonary surfactant through dilution and direct inhibition, along with damage of type II alveolar pneumocytes; (4) vasoconstriction of pulmonary vessels resulting in pulmonary hypertension and persistence of fetal circulation, that *in vitro* studies indicate are not due to the direct vasoconstrictor effect of meconium but possibly to the release of agonist humoral factors, such as thromboxane or TNFα, by lung parenchyma capable of enhancing the pulmonary arterial constrictor potential [6].

Inflammation plays a major role in the pathogenesis of MAS, and pulmonary function improves concomitantly with resolution of inflammation. Pro-inflammatory substances in meconium can induce lung inflammation in MAS directly via cytokines and heme present in meconium, and indirectly by inducing cytokine release from epithelial cells and alveolar macrophages. IL-1β, IL-6, and IL-8 appear to be expressed at high levels in early postnatal life of infants with MAS, making it highly likely that these cytokines are already expressed in utero before birth supporting the hypothesis of perinatal timing of meconium aspiration in most cases of MAS [7].

59.3 Prevention Strategies

As aforementioned, a reduction of the incidence of MAS over the past 3 decades is probably due to better prenatal care, leading to a reduction of postterm pregnancies and better fetal surveillance before and during labor of high-risk fetuses such as those with intrauterine growth restriction. Interventions of some efficacy to further decrease the incidence of MAS could probably be done only at a prenatal or perinatal stage, removing causes of acute or above all chronic fetal asphyxia. Indeed, the early postnatal management of a MSAF delivered infant has progressively been limited to non "vigorous" babies, assuming that intubation and tracheal suctioning of an already compromised newborn can reduce the incidence or at least the severity of MAS. This point has yet to be demomstrated. It is still unclear whether obstruction of airways caused by aspiration of meconium has a pivotal role in the progress of MAS. Antepartum, peripartum and postpartum interventions to remove meconium from the newborn's airway in order to prevent its aspiration in the presence of MSAF have been evaluated over the past 3 decades, including (1) amnioinfusion, (2) suctioning the infant before the first breath, and (3) intubation and tracheal suctioning of the infant immediately after delivery. Amnioinfusion has been proposed for interventions when monitoring detects variable fetal heart. Potential mechanisms through which amnioinfusion could act include mechanical cushioning of the umbilical cord, which could correct or prevent recurrent umbilical compressions, and dilution of meconium that could reduce its mechanical and inflammatory effects in the pathogenesis of MAS. It would appear that in settings where electronic

fetal monitoring is routinely used, amnioinfusion confers no additional benefit in terms of prevention of MAS while in settings without these capabilities, often in developing countries, amnioinfusion has been protective with improved neonatal outcomes, reduced MAS (4% versus 18%) and improved maternal outcomes [8]. ACOG Committee on Obstetric Practice has recently recommended that "routine prophylactic amnioinfusion for the dilution of MSAF should be done only in the setting of additional clinical trials. However, amnioinfusion remains a reasonable approach in the treatment of repetitive variable decelerations, regardless of amniotic fluid meconium status" [9].

Intrapartum suctioning, combined with intubation after delivery of a newborn from MSAF, has been considered standard procedure for more than 25 years. The aim is to clear as much meconium as possible from the airway before the infant is able to take a breath. Recent evidence has argued against the efficacy of intrapartum oropharyngeal and nasopharyngeal suctioning of meconium using a DeLee suction catheter before delivery of the shoulders (intrapartum suctioning). In a multicenter, randomized controlled trial involving 2,514 full-term women with MSAF, Vain and colleagues failed to show a benefit of intrapartum suctioning on the need for endotracheal intubation, incidence of MAS, need for mechanical ventilation, and neonatal mortality [10]. Based on this evidence, the ACOG Committee on Obstetric Practice recently recommended that "infants with MSAF should no longer receive intrapartum suctioning" [11].

Quite recent evidence has also argued against the efficacy of intubating and suctioning all infants born through MSAF. A number of studies showed no benefit in tracheal suctioning of "vigorous" infants. The largest international prospective randomized controlled trial to assess a selective approach is that conducted by Wiswell and colleagues [4] showing no difference in the rate of MAS in infants who were intubated (3.2%) or not (2.7%). There was no difference between the groups in subanalyses that adjusted for the thickness of the meconium in the amniotic fluid. Subsequent to this publication, the Neonatal Resuscitation Program (NRP) changed its recommendations and advised that apparently "vigorous" meconium-stained infants do not need intubation and intratracheal suctioning. A future question that needs to be answered is whether or not depressed meconium-stained infants actually benefit from having their airways intubated and suctioned. As many clinicians believe that most cases of MAS are due to in utero aspiration of MSAF, the potential efficacy of tracheal cleansing needs to be assessed in a well-conducted randomized, controlled trial, while some have suggested an even more aggressive approach: endoscopic in-utero suctioning of the fetus before delivery [12]. However, this procedure has yet to be tested and proven, and therefore cannot be recommended.

Current recommended delivery room management of the meconium stained newborn is that, if meconium is present, and the newborn is depressed or not "vigorous", the clinician should intubate the trachea and suction meconium from beneath the glottis. Thus, a skilled resuscitation team should be present at all deliveries that involve MSAF. If the delivered infant is judged as "vigorous", that is if he or she has (1) strong respiratory efforts, (2) good muscle tone, and (3) a heart rate more than 100 beats/min, there is generally no need for tracheal suctioning, and the pediatrician may procede with routine management. When an infant is not "vigorous", the goal is to clear the airway as quickly as possible to minimize the amount of meconium aspirated. The infant may be given free-flow oxygen and placed under a radiant heater, but drying and stimulating should be delayed. At this point, direct laryngoscopy should be performed with suctioning of the mouth and hypopharynx (with a 12F or 14F suction catheter) under direct visualization, followed by intubation and then applying suction (approximately 100 mmHg) directly to the endotracheal tube while it is slowly withdrawn. The process is repeated until either "little additional meconium is recovered, or until the baby's heart rate indicates that resuscitation must proceed without delay". Should positive pressure ventilation be required before complete airway clearance, a suction catheter inserted through the tracheal tube may be used to continue meconium removal [13].

59.4 Clinics and Therapy

Most infants born through MSAF require no interventions, however it is important to monitor these infants closely for signs of respiratory distress that possibly develops within the first 12 hours of life.

A severely compromised neonate born with MSAF should not be a priori labeled as a case of MAS until other causes of neonatal compromise have been excluded. Severe MAS should cease to be a diagnosis of inclusion (any respiratory distress in the context of MSAF with any radiologic finding), but rather become a diagnosis of exclusion. Its diagnostic criteria should be strict (i.e., presence of meconium in the mouth or trachea at delivery, early onset of respiratory distress, and chest X-ray evidence of "patchy" areas of opacification or pulmonary air leak) and limited to cases without infection or chronic asphyxia. Infants at risk of MAS who show signa of respiratory distress (tachypnea, cyanosis, retractions, grunting, rales and rhonchi at chest auscultation, barrel shaped chest) must be transferred to the neonatal intensive care unit. An infant developing a severe MAS will require a wide range of intensive therapies to be rapidly available and aimed at increasing oxygenation while minimizing the barotraumas, that may lead to air leak syndromes: intubation and endotracheal suction in the delivery room, mechanical ventilation (conventional or high frequency ventilation) preferably already started in delivery room (to avoid bag ventilation at higher risk of determing air leaks), placement of a central catether in the umbilical vein, sedation with continuous iv infusion of

fentanyl and/or midazolam, administration of endotracheal surfactant as bolus or lavage, inhaled nitric oxide (iNO) if persistent pulmonary hypertension is present, inotropic drugs and/or plasma transfusion to treat hypotension, antibiotic prophylaxis (as sepsis is often in the differential diagnosis), anti-inflammatory drugs to reduce lung inflammation, strict evaluation and management of acidosis, electrolyte imbalance and hypoglycaemia. Severe case of MAS may need for extracorporeal membrane oxygenation (ECMO), even if the percentage of newborns requiring ECMO has decreased since the introduction of treatment of persistent pulmonary hypertension with iNO. A chest X-ray will be performed to evaluate if radiographic features are compatible with the diagnosis of MAS and to exclude the presence of pnemothorax, pneumomediastinum or pulmonary interstitial emphysema, which frequently complicate MAS. Roentgenographic findings may include coarse irregular or nodular pulmonary densities, areas of diminished aeration or consolidation (atelectasis) alternating with areas of hyperinflation and generalized hyperinflation. The classic findings in MAS are described as diffuse, asymmetric patchy infiltrates, but because of the diverse mechanisms that cause disease, various radiographic findings may be present. Radiographic clearing is slow over a period of days or weeks if classic radiographic findings of MAS are present. A two-dimensional echocardiogram will be performed to exclude a congenital heart defect and to assess pulmonary hypertension, myocardial contractility and cardiac output, intracardiac right to left shunts, and shunt through the ductus arteriosus. Usually, in cases of MAS, early blood tests are characterized by hypoxemia, metabolic acidosis, mild to severe hypercarbia, and no signs of infection (C reactive protein negative, no leucocytosis or leucopenia). This kind of biochemical pattern prevents the physiological drop of pulmonary arterial resistance with increase in pulmonary blood flow, resulting in right-to-left shunts at atrial and ductus arteriosus level, thus maintaining and further reducing hypoxemia into a vicious cycle. Leucopenia may present later as a consequence of the accumulation of polymorphonuclear leukocytes into the lung tissues.

Cleary and Wiswell have proposed criteria to define MAS severity: (1) mild MAS is disease that requires less than 40% oxygen for less than 48 hours, (2) moderate MAS is disease that requires more than 40% oxygen for more than 48 hours with no air leak, and (3) severe MAS is disease that requires assisted ventilation for more than 48 hours and is often associated with persistent pulmonary hypertension [1]. Pneumothorax is still a frequent complication of MAS (8–20%), and it is an important indicator of a poorer prognosis. Approximately 40% of the babies with MAS require mechanical ventilation, about 1.4% of ECMO. ECMO use has decreased during the last decade, while high frequency oscillatory ventilation (HFOV) and iNO have been used with increasing frequency [2]. However, no prospective, randomized, controlled trials have compared conventional ventilation versus HFOV in MAS. Theoretically, high-frequency ventilators should reduce airleak syndromes in MAS, but animal and clinical models have yielded conflicting results. High-frequency ventilators may slow the progression of meconium down the tracheobronchial tree and allow more time for meconium removal [14].

Inhibition of surfactant function in the alveolar space is an important element of the pathophysiology of the disease, thus representing the rationale for administration of exogenous surfactant preparations in MAS, initially as standard bolus therapy and, more recently, in association with therapeutic lung lavage. In infants with MAS, surfactant administration may reduce the severity of respiratory illness and decrease the number of infants with progressive respiratory failure requiring support with ECMO [15]. Bolus surfactant should be administered as early as practicable to infants who exhibit significant parenchymal disease, at a phospholipid dose of at least 100 mg/kg, rapidly instilled into the trachea. Lung lavage with dilute surfactant has recently emerged as an alternative to bolus therapy in MAS, which has the advantage of removing surfactant inhibitors (hemoglobin, plasma proteins) from the alveolar space in addition to augmenting surfactant phospholipid concentration. The ideal fluid volume and technique for lavage in MAS are not yet clear; experimental data would suggest a total lavage volume of 30 mL/kg (administered as two 15 mL/kg aliquots) at a 5mg/mL phospholipid concentration (lower concentrations make surfactant more sensitive to inactivation) as most effective in improving oxygenation and pulmonary mechanics, and in attenuating lung injury. Lesser total lavage volumes or smaller aliquot volumes removed meconium less effectively, whereas a greater total lavage volume resulted in unacceptably high deposition of aqueous fluid in the lung. The advised procedure would be the following: a) adequate stabilisation of the newborn, b) sedation and muscle relaxation to minimise bradycardia and maximise fluid recovery, c) perform lavage in a rapid sequence, with instillation and recovery of each lavage aliquot within 60–80s, d) use open suction with the ventilator disconnected and manual vibratory chest squeezing to collect as much fluid as possibile, and e) ventilate with higher mean airway pressure (2–4 cmH_2O more) than the pre-lavage one for 30 minutes to re-recruit lung volume and clear retained lavage fluid. As large-volume lavage procedure is inevitably associated with a fall in oxygenation during and for a short time after lavage, it needs to be included in randomised controlled trial of lavage therapy in ventilated infants with severe MAS in order to assess the real benefits against the potential risk associated with the procedure [16]. Thus, this procedure cannot be routinary recommended.

MAS is frequently accompanied by persistent pulmonary hypertension. iNO causes selective pulmonary vasodilation by acting directly on the vascular smooth muscle. By dilating the blood vessels in well-ventilated areas of lung, iNO decreases the ventilation perfusion ratio mismatch and improves oxygenation in infants with persistent pulmonary hypertension. Proper administration of iNO requires adequate

delivery to the alveoli, thus it may be less effective in patients with MAS, in part because of the physical "barrier" to its diffusion across the alveolar membrane produced by the meconium. Pretreatment with surfactant seems to aid the delivery of iNO to alveoli, with a resultant increase in oxygenation [17]. Kinsella and colleagues showed that the combination of HFOV and iNO may be more successful than either treatment alone in patients with MAS, likely due to improved lung inflation and better delivery of the drug [18]. iNO appears to improve outcome in hypoxemic term and near-term infants by reducing the need for ECMO, but has no apparent effect on mortality [19].

Since the introduction of treatment of persistent pulmonary hypertension with iNO, the need for ECMO has decreased. However, about 40% of infants with MAS treated with iNO fail to respond and require bypass. ECMO is still considered an important tool in treating the newborns with MAS and intractable respiratory failure, with the highest survival rate for any neonatal condition suitable for ECMO (on the order of 93–100%). Despite the evidence pointing to the efficacy of ECMO in severe MAS, without causing excess pulmonary or neurological morbidity (because of prolonged hypoxia), some infants are still referred late for ECMO. Recent introduction of new therapies for respiratory failure in the newborn with MAS such as iNO, surfactant, and HFOV may postpone the use of ECMO. A delay in ECMO initiation longer than 96 hours results in a significant increase in duration of ECMO and length of post-ECMO ventilation, with a significant increase in mortality in this group of infants. Thus, the need of ECMO in severe MAS should be predicted as early as possibile to avoid ventilator-associated lung injury and to improve outcome [20].

Other potential therapeutic interventions for treating MAS have been indicated: synthetic surfactant, captopril, tezosentan, pentoxifylline. Some surfactants may be more resistant to inactivation by meconium [21], however the search for new synthetic surfactant preparations that are highly resistant to inactivation by meconium or other forms of toxic pneumonitis is ongoing.

In MAS inflammation plays an important role in the pathogenesis of lung injury. A number of anti-inflammatory agents have been investigated for their therapeutic values, such as corticosteroids, pentoxifylline, and aminophylline. A Cochrane systematic review of available clinical trials concludes that there is insufficient evidence on the use of systemic steroids in the treatment of MAS [22]. In a recent randomised controlled study, however, both intravenous methylprednisolone and nebulised budecort were able to suppress TNFα level in the tracheal aspirate of infants with MAS. Treated infants also had a shorter hospital stay, shorter duration of oxygen dependency, and earlier radiological clearance of the lung fields [23]. Local corticosteroid administration, both nebulised or intracheally administered (budenoside), resulted in the same benefits with lower adverse effects than systemic corticosteroid; thus further testing of local corticosteroid administration is worthwhile. Pentoxifylline is a methylxanthine derivative and phosphodiesterase inhibitor. It is categorised as an anti-inflammatory agent because of its anti-inflammatory action in lung tissues and its ability in suppressing neutrophil action and proinflammatory cytokines in the injured lung. No study on the use of pentoxifylline in treating MAS in human neonates has been reported. Animal studies indicate that pentoxifylline administration effectively suppresses the meconium-induced increase in alveolar macrophage number and local TNF-α production in the insulted lungs and early treatment of MAS with pentoxifylline could be a new, pathophysiologically plausible therapeutic alternative [24]. Aminophylline, another methylxanthine derivative, has also demonstrated a therapeutic potential in the treatment of MAS in the animal model, effectively decreasing intrapulmonary shunting, lung edema and recruitment of neutrophils, thus enhancing oxygenation in meconium-instilled rabbits [25].

In acute lung injury following meconium aspiration pulmonary hypertension is related to an increase of endothelin (ET)-1 levels. Recently tezosentan, an intravenous dual ETA and ETB receptor antagonist, has been shown to improve pulmonary gas exchange and hemodynamics by decreasing mean arterial pulmonary pressure and pulmonary vascular resistance in experimental meconium aspiration. Tezosentan thus seems to act predominantly in the pulmonary vasculature, the site of turnover of ET-1, and its effect to be enhanced by the combination with an inhalational drug like iloprost [26].

Cell death by apoptosis in the lungs has been demonstrated in MAS. Meconium-induced apoptosis is activated by a strong vasoconstrictor, angiotensin II, which is the product of conversion of angiotensin I by angiotensin-converting enzyme (ACE) within the cell. Pretreatment with captopril, blocking the conversion of angiotensin I to the apoptotic-inducer angiotensin II, significantly inhibits meconium-induced lung apoptosis and also reduces mortality of rabbit pups from meconium. Captopril could represent another potential new therapeutic intervention for MAS [27].

59.5 Conclusions

More efficient modalities of treatment have greatly improved survival rate of MAS infants over the last three decades, but the amount of morbidity among survivors seems unchanged and growing concern exists for long-term poor neurodevelopmental and pulmonary sequelae of infants even asymptomatic at the time of discharge from the nursery.

The American Academy of Pediatrics Neonatal Resuscitation Program Steering Committee has promulgated guidelines for management of the baby exposed to meconium. The guidelines are under continuous review and are revised as new evidence-based research becomes available [13].

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60

Molecular Structure of Surfactant: Biochemical Aspects

Tore Curstedt

60.1 Introduction

Oxygen is needed for energy production in the living organism and is a prerequisite of life. This oxygen, which is taken up from the air during breathing, has to pass from the alveoli to the blood in order to be transported to different organs where it can participate in the metabolic processes in the body. The passage from the alveoli to blood is crucial and is dependent on a barrier composed of the alveolar wall, basal laminae and the endothelial cells. Exposure of a large alveolar surface to the air is required to facilitate an appropriate gas exchange in order to fulfill the energy needs of the living organism.

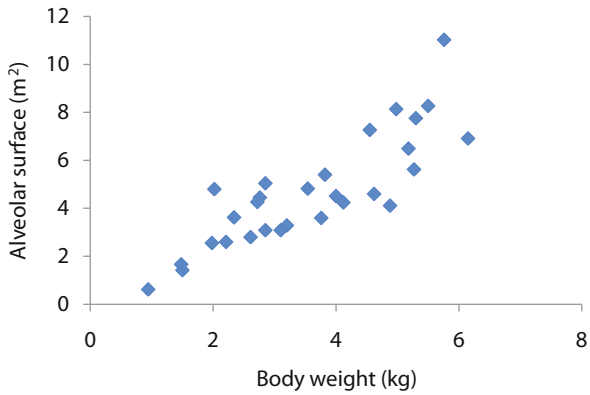

Fig. 60.1 Correlation between body weight and total alveolar surface area. The values are taken from [1]

60.2 Alveolar Surface

The development of the alveoli starts in the weeks before birth and the alveolar area increases roughly linear with body weight (Fig. 60.1). At birth the alveolar area is 3–5 m^2 at end-expiration and about 80 m^2 in adults [1, 2]. Thus, the area is about 1 m^2/kg body weight. The number of alveoli increases in a curvilinear fashion with up to 150 million at birth, which is half to one third of the number in adults.

The alveoli are lined with a surface-active material, called surfactant. This material facilitates alveolar increase during inspiration and prevents alveolar collapse at end-expiration. Thus, pulmonary surfactant is essential for normal lung function and must fulfill properties such as rapid film formation through adsorption from the hypophase, low surface tension at end-expiration and effective replenishment of the surface film during inspiration.

The surfactant lines the alveoli as a monolayer in combination with areas of multilayers [3]. The amount of surfactant needed for formation of a monolayer at end-expiration is about 3 mg/m^2 indicating that a newborn term infant needs 10 mg surfactant for the monolayer. However, during inspiration the alveolar area will increase and some of the surfactant is continuously metabolized. Thus, there must be a surplus of surfactant in the lungs and the pool in newborn full-term animals has been calculated to be about 100 mg/kg body weight [4]. The alveolar subphase beneath the surfactant film has been shown in rat lungs to have an average thickness of about 0.2 μm [5]. If humans have a similar thickness the total aqueous alveolar phase would be about 0.2 mL/m^2 alveolar surface area. This indicates that term infants may have a mean alveolar surfactant concentration of about 500 mg/mL presuming that newborn term infants have about the same surfactant pool size as animals.

60.3 Isolation of Pulmonary Surfactant

The knowledge about the composition of pulmonary surfactant has usually been obtained from lung lavage. The lungs

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

are lavaged using isotonic sodium chloride solution, followed by fractional centrifugation of the lavage fluid. In the first centrifugation, using 300–400 g for 10 min, cell debris is removed and the supernatant is then centrifuged at 10,000 g for 20 min. The pellets obtained contain a highly surface-active material composed of lipid-protein aggregates that are enriched in tubular myelin and lamellar bodies. Lamellar bodies are stored in type II epithelial cells and are secreted by exocytosis into the alveolar lumen and converted to tubular myelin, which is the source for the surface-active monolayer lining the alveolar surface.

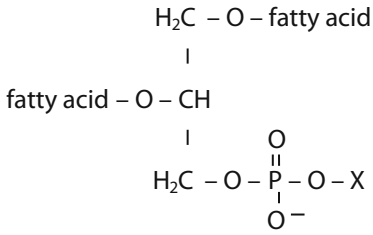

Fig. 60.2 General structure of phospholipids. X: e.g. choline, ethanolamine, glycerol, serine, inositol

60.4 Composition of Surfactant

Pulmonary surfactant lining the alveolar surface is a complex mixture of lipids and proteins. It is composed of about 80–85% phospholipids, 8–10% neutral lipids and 8–10% proteins [6, 7] (Table 60.1). Cholesterol is the main neutral lipid but trace amounts of triglycerides and free fatty acids are also present. Other components such as complex carbohydrates and glycolipids are also found in surfactant.

60.4.1 Phospholipid Composition

The phospholipids are mainly responsible for reducing the surface tension at the air-liquid interface. Its composition is complex and at least fifty different phospholipids may be found in surfactant [8]. The most common structure is a glycerol skeleton with two ester-linked fatty acids and a phosphate group (Fig. 60.2). Different components, e.g., choline, ethanolamine, glycerol, serine or inositol are usually bound to the phosphate group, thus making up the different classes of phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine and phosphatidylinositol. Depending on the fatty acid composition, each phospholipid class is composed of many molecular species. About 80% of the phospholipids consist of phosphatidylcholine with its surface-active disaturated species. These disaturated species, containing 40–60% of total phosphatidylcholines are dominated by dipalmitoylphosphatidylcholine, a phosphatidylcholine containing two palmitic acids. Although phosphatidylcholines predominate among the phospholipids, phosphatidylglycerol is present in amounts up to 10% and smaller amounts of phosphatidylethanolamine, phospatidylserine and phosphatidylinositol are also present.

At the air-liquid interface lining the alveoli the phospholipids are oriented in a monolayer with the hydrophilic head groups towards the aqueous phase and the hydrophobic acyl groups towards the air [9].

A high concentration of phospholipids, mainly dipalmitoylphosphatidylcholine, at the interface diminishes the number of water molecules exposed to the air resulting in a lower alveolar surface tension and less force required to open up the alveoli during inspiration. This saturated phospholipid, in contrast to unsaturated species, can be packed to a very high density at the air-liquid interface and provides a large reduction of surface tension during expiration, thus preventing the alveoli from collapse at end-expiration. However, the melting point of dipalmitoylphosphatidylcholine bilayers is 41°C, which is above the physiological temperature of the human body while that of unsaturated species is much lower. Thus, the presence of significant amounts of unsaturated phospholipids in surfactant reduces the melting temperature of the monolayers to values lower than 37°C, which is important for the physiological properties of pulmonary surfactant. Resent results indicate that cholesterol, which constitutes 5–10% of pulmonary surfactant, may modulate the structure of surfactant membranes by decreasing the packing and increasing the mobility of phospholipids in the monolayer.

Table 60.1 Composition of pulmonary surfactant

Component	%
Dipalmitoylphosphatidylcholine	30–40
Other disaturated phosphatidylcholines	3–5
Unsaturated phosphatidylcholines	35–40
Phosphatidylglycerol	5–10
Other phospholipids	5–10
Neutral lipids (mainly cholesterol)	5–10
Hydrophobic surfactant proteins (SP-B, SP-C)	1–4
Hydrophilic surfactant proteins (SP-A, SP-D)	4–8

60.4.2 Surfactant-Associated Proteins

Proteins represent less than 10% of the total mass of pulmonary surfactant. Four different surfactant-associated proteins have been identified. The two hydrophobic surfactant proteins, SP-B and SP-C, increase the adsorption and spreading of the surfactant film at the alveolar air-liquid interface [7] and are crucial for obtaining a functional surfactant. The two hydrophilic surfactant proteins, SP-A and SP-D, have a primary role in host defense [10].

60.4.2.1 Surfactant Proteins A and D

SP-A and SP-D belong to a larger family of proteins termed collectins containing both collagenous and lectin domains. The lectin domain mediates the calcium-dependent carbohydrate binding and is also called carbohydrate recognition domain. The basic structural unit of collectins are trimers which are composed of three similar or identical polypeptides. These trimers then multimerize resulting in differently shaped multimeric forms.

SP-A is the most abundant protein in surfactant. Each monomer with a molecular mass of about 30–36 kDa contains four structural domains with a short N-terminal segment containing interchain disulfide bonds, a collagenous region, a hydrophobic neck region and a carbohydrate recognition domain. Three monomers form a trimer by association of the collagenous and neck regions. Six trimers form the octadecameric molecule, which is stabilized by disulfide linkages in the N-terminal regions. This octadecameric molecule forms a bouquet-like structure with an apparent molecular mass of about 650 kDa. The protein is closely associated with the phospholipids in the presence of calcium ions and together with SP-B and phospholipids tubular myelin is formed.

SP-D is composed of monomeric subunits, each with a molecular mass of about 43 kDa. Each monomer possesses similar domains as that of SP-A. SP-D usually forms a cruciform dodecamer assembled from four trimers with a molecular mass of about 520 kDa. However, SP-D can assemble into even higher-order multimers when dodecamers assemble into "fuzzy-ball" structures. SP-D binds to phosphatidylinositol and a variety of glycolipids, which are minor components of surfactant. However, only a small part of SP-D co-isolate with surfactant lipids.

SP-A and SP-D are synthesized and secreted by alveolar type II cells and by Clara cells but SP-D especially is also expressed in other tissues such as gastric and intestinal mucosa. Both proteins interact with a variety of pathogens such as bacteria, viruses and fungi and protect the lungs from infection by aggregation of pathogens, stimulation of phagocytic activity and modulation of inflammatory response. SP-A does not seem to have any effect on spreading of surfactant phospholipids but the protein enhances the resistance to inactivation of surfactant by proteins, e.g., albumin. It has also been shown that SP-A deficient mice survive and breed normally but they lack tubular myelin and have increased susceptibility for infections.

60.4.2.2 Surfactant Proteins B and C

The hydrophobic surfactant proteins SP-B and SP-C are important for surfactant function. SP-B is expressed in alveolar type II cells and Clara cells while SP-C is expressed only in type II cells. The proteins are structurally different but they are both required for sustaining respiratory physiology by decreasing surface tension of the alveolar air-liquid interface and to prevent the alveoli from collapse at end-expiration. Mutations in the SP-B gene have been shown to lead to lethal respiratory failure at birth [11]. However, these mutations also interact with SP-C processing by inhibiting the formation of mature SP-C from its proform. Also abnormalities in surfactant phospholipids have been observed in SP-B deficient infants. The pathophysiology of lung disease associated with mutations in the SP-C gene is incompletely understood. These mutations are usually linked to lung disease in older children and adults. Also heterozygotes have a lack of mature SP-C leading to alveolar instability with atelectasis, inflammation and fibrosis.

SP-B has 79 amino acid residues with three intramolecular disulfide bridges and one intermolecular disulfide bridge, thus forming a dimer. This dimer may interact with the surface of lipid bilayers by means of four or five amphipatic α-helices in each monomer. SP-B belongs to the saposin family of proteins, in which all members interact with lipids [7]. However, in contrast to the other members of the saposin family, SP-B is permanently associated to the membranes, which is probably due to its high hydrophobicity. The three-dimensional structure of SP-B has not been determined, but SP-B differs from the other saposins by being a covalent dimer not soluble in water. The dimeric structure of SP-B may account for its ability to cross-link juxtaposed lipid membranes, thus catalyzing the transfer of phospholipids from surfactant membranes into the interfacial surface-active film. The protein has a net positive charge which may promote a selective interaction with anionic phospholipids, especially phosphatidylglycerol, but clear evidence about that is missing. SP-B is critical for formation of lamellar bodies and is required for formation of tubular myelin.

SP-C is a small very hydrophobic lipopeptide of 35 amino acid residues with two palmitoylated cysteines in the N-terminal part [7]. It consists of a very regular and rigid α-helix covering more than 70% of the molecule and an unstructured N-terminal part. The α-helical segment adopts a transmembrane localization orientated near parallel to the phospholipid acyl groups. The length of the helix is in very good agreement with the thickness of a fluid dipalmitoylphosphatidylcholine bilayer. The primary translation product of human SP-C is a proform of 197 amino acid residues. Mutations in the C-terminal part of the molecule, not involving the mature SP-C, are seen in patients with interstitial lung diseases. These mutations may result in a conversion of the proSP-C from the native structure to β-sheets found in amyloid-like fibrils [12]. These fibrils are thought to be cell toxic and may give rise to organ malfunction and disease.

60.5 Structure of Pulmonary Surfactant

The surfactant components are synthesized in alveolar type II cells and secreted as lamellar bodies into the alveolar subphase. The lamellar bodies are transformed into tubular

myelin whereupon the material adsorbs quickly to the alveolar air-liquid interface forming a surface-active film. The thin aqueous phase covering the alveolar surface contains multiple membrane structures, including lamellar bodies, tubular myelin and the surface-active film. The air-liquid interface is necessary for respiratory gas exchange and the alveoli must be able to open during inspiration without collapsing during end-expiration. These criteria may be fulfilled with a complex phospholipid mixture which can be packed to a very high density at the air-liquid interface and have a melting point below the physiological temperature of the human body. For spreading the phospholipids during breathing the hydrophobic proteins SP-B and SP-C are necessary. The interface should also be a defense against different pathogens and for this reason native surfactant contains the hydrophilic proteins SP-A and SP-D.

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Surfactant Metabolism in Neonatal Lung Diseases

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61.1 Background

Pulmonary surfactant reduces surface tension in the lungs to impressive low values. When secreted by the type II cells into the thin liquid layer that lines the alveolar air spaces, vesicles of pulmonary surfactant absorb readily to the air water interface and form an interfacial film. The surfactant unique tensioactive properties were originally explained by its unique phospholipid composition, which is strikingly comparable across species [1]. Of the surfactant lipids, 80–90% are phospholipids, of which phosphatidylcholine (PC) is quantitatively the most important, accounting for 70–80% of the total. Other lipids include phosphatidylglycerol (PG), phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin, cholesterol, triacylglycerols, and free fatty acids. Approximately 60% of the PC contains two saturated fatty acids (DSPC) of which dipalmitoyl (16:0/16:0; DPPC) is the most abundant. DPPC and PG are key phospholipids that permit normal breathing by reducing surface tension to near zero at the end of the respiratory cycle.

More recent studies have suggested that the hydrophobic proteins SP-B and SP-C contribute to the stabilization of the phospholipid film. Four surfactant proteins have so far been identified [2]. SP-A and SP-D are hydrophilic, and SP-B and SP-C are hydrophobic. They are either exclusively lung associated or predominately found in the lung. SP-A is the most abundant surfactant protein and is essential for the formation of tubular myelin. It functions as a regulator of phospholipid insertion into the monolayer, and modulates the uptake and secretion of phospholipids by type II cells. However, mice that lack SP-A indeed have no tubular myelin but have normal lung function and surfactant metabolism even during exercise [3]. Furthermore, together with SP-D, SP-A has an important role in lung defense [4]. SP-A and SP-D bind pathogens and facilitate their clearance [4]. The absence of SP-D results in increased surfactant lipid pools in the airspaces and emphysema in lungs of mice [5]. SP-B plays a role in formation of tubular myelin and together with SP-C it promotes rapid phospholipid insertion into the air-liquid interface. SP-B also influences the molecular ordering of the phospholipid layer. Infants with a genetic absence of SP-B develop lethal respiratory distress after birth, which can only be treated by lung transplantation [6]. Absence of SP-B causes a loss of lamellar bodies, tubular myelin, and an incompletely processed SP-C [7]. SP-C regulates the phospholipid ordering in the monolayer, enhances the reuptake of surfactant lipids *in vitro*, and may have a role in surfactant catabolism. Mice that lack SP-C have normal surfactant and lung function, and have no abnormalities in SP-B processing [8]. Interestingly the alveolar pool of surfactant in newborn infants with respiratory distress syndrome (RDS) does not differ much from the alveolar pool of adults without respiratory disease [9].

61.2 Surfactant Kinetics: Animal Studies

Surfactant PC is synthesized from phospholipid precursors (e.g., fatty acids, glycerol, choline, glucose) in the Golgi apparatus [10]. In the fetal type II cell, intracellular glycogen stores appear to be a major source of the glycerol backbone of PC, whereas in the adult lung glucose from the circulation is a major substrate. Choline is mainly derived from the diet. The fatty acids of surfactant phospholipids are synthesized de novo in the type II cell, or taken up from the blood, or are derived from recycling of alveolar surfactant phospholipids [10].

Alveolar surfactant can be cleared by different pathways. Surfactant components can be reutilized through uptake by the type II cell, incorporation into the lamellar bodies and then direct re-secretion [11]. Another way is recycling of degraded surfactant components to synthesize new surfactant lipids or proteins. Finally, surfactant can be removed from the

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

lung, either as intact molecules or as degraded products [12]. The efficiency of recycling is age dependent, and has been calculated to be 90% in young pigs [13], >90% in the newborn rabbit and 50% in adult rabbits [14].

The time required for de novo PC synthesis, secretion, and significant alveolar accumulation has been studied in animals using radioactively labeled substrates [15]. Compared to adult animals, the time to reach peak specific activity is longer in term newborns [16]. Preterm ventilated lambs show a slow movement of surfactant PC from the synthesis sites to the alveolus similar to term ventilated lambs [17].

61.3 Surfactant Kinetics: Human Studies

Recently, stable isotope techniques were used to study surfactant metabolism in human infants. Labeled precursors were infused intravenously and incorporation of label was measured by mass spectrometry in surfactant PC or DSPC isolated from tracheal aspirates.

In preterm infants with RDS who received a 24-hour intravenous infusion of the stable isotope [U-^{13}C]glucose, the palmitic acid of surfactant PC became labeled approximately about 17 h and was maximally labeled after ~75 h [18, 19]. By using other precursors such as [U-^{13}C]palmitic acid, [U-^{13}C]linoleic acid, and [1-^{13}C]acetate to study surfactant kinetics, the same results were estimated [20, 21].

In term ventilated neonates without significant lung disease using infusion of [U-^{13}C]glucose, [U-^{13}C]palmitic acid or [1-^{13}C]acetate as precursors, the first appearance of label in surfactant PC was after ~9 h from the start of the infusion, and the maximal enrichment at ~44 h [21–23]. As in animal studies, these results of human stable isotope studies show slower surfactant kinetics in preterm infants compared to term infants. The kind of precursor does not affect the results. The fractional synthesis rate (FSR) of surfactant after infusion of labeled glucose is a measure of the percentage of surfactant that is synthesized from glucose per day. In premature infants who received a 24-hour [U-^{13}C]glucose infusion, the FSR was calculated to be ~4%/day [18, 19]. Different values were found for different precursors: preterm infants who received labeled palmitic acid and linoleic acid had an FSR of ~12 and ~25%/day, respectively [24]. FSR in preterm and term infants after infusion of labeled acetate was ~2 and ~15%/day, respectively [19]. When term infants received labeled palmitic acid, the FSR was found to be ~17%/day [22]. After infusion with labeled glucose, term infants had a FSR of ~8%/day [23]. These data show a lower FSR in preterm infants compared to term infants.

The slow secretion and alveolar accumulation of surfactant are balanced in the term and preterm lungs by slow catabolism and clearance. For example, the half-life of radiolabel surfactant phospholipids given endotracheally to term lambs is about 6 days [24]. However, in premature baboons using the same method the half-life is about 30 h [25], which may be due to more recycling in premature animals, and to species difference. Half-lives calculated in animal studies differ depending on gestational age, postnatal age, the labeled substrate used, and the surfactant pool studied.

Half-life measurements of disappearance of labeled surfactant in human infants have been performed mainly in preterm infants with RDS. Half-lives were longer when disappearance of label in surfactant was measured after the intravenous use of labeled precursors: the half-life of ^{13}C-labeled PC-palmitate measured in tracheal aspirates of preterm infants with RDS after labeled glucose infusion was ~96 h [18, 19, 26]. In preterm infants the surfactant half-life after infusion of [U-^{13}C]palmitic acid, [U-^{13}C]linoleic acid or [1-^{13}C]acetate was ~98, ~47, and ~106 h, respectively [21, 27]. Using [U-^{13}C]palmitic acid, [U-^{13}C]glucose, or [1-^{13}C]acetate in term infants, the half-life was ~43, ~63, and ~28 h, respectively [21–23]. Thus, surfactant half-life is different between preterm and term infants, with a lower disappearance of label in the preterm infants reflecting slower surfactant kinetics, especially in RDS.

There are two studies in the literature in critically ill term infants who were studied using different metabolic precursors for surfactant metabolism [21, 27].

Cogo and colleagues showed marked patient differences in PC kinetics after infusing labeled fatty acids in a group of critically ill infants with various diagnoses [27].

Bohlin and colleagues reported that term infants with primary respiratory failure had a surfactant metabolism similar to that of preterm infants with RDS, suggesting either delayed maturity of the surfactant system or disruption from the underlying disease [21].

In summary, in human studies using stable isotopes the surfactant PC metabolism is even slower in preterm infants with RDS compared to term infants without lung disease. Moreover, term infants with respiratory failure have abnormal surfactant metabolism, which resembles that of preterm infants with RDS.

61.4 Surfactant Pool Size

In most species studied, PC, and DSPC in particular, increase in the last trimester of pregnancy [28]. In amniotic fluid, surfactant concentrations increase, reflecting accumulation of alveolar surfactant. This increase is also reflected in an increasing lecithin/sphingomyelin (L/S) ratio [29].

During and shortly after birth large amounts of surfactant are released into the alveolar space [30]. Since there is no depletion of the intracellular surfactant pool, this increase in alveolar pool shortly after birth is accompanied by an increase in de novo surfactant synthesis. However, incorporation studies, as mentioned above, showed slow surfactant metabolism, which means that it takes a long time before de novo synthesized surfactant is detectable in the alveolar

space. Increased lung tissue and alveolar surfactant pools can only be explained by the rapid mobilization of surfactant from other pools (lamellar bodies, small vesicles) within type II cells. The amount of lung tissue and alveolar surfactant changes with age. Surfactant pools are very high in the newborn period in all species studied to date and decrease subsequently with lung maturity. In humans total amounts of sat PC in alveolar washes at autopsy also decrease with age [9]. In preterm infants with RDS, surfactant pool sizes in the alveolus are low (2–10 mg/kg) [16]. Total lung DSPC in the human adult, at autopsy, was ~22 mg/kg, and ~2 mg/kg in alveolar wash [9]. In human infants surfactant pool size can be estimated *in vivo* by measuring the dilution of a labeled surfactant component [31].

Hallman and colleagues [32] and Griese and colleagues [33] showed an apparent phospholipid pool size of ~16 mg/kg in human preterm neonates with RDS, by using PG as label to measure surfactant pool size. Pool size measurements in preterm infants with RDS using endotracheally administered stable isotope (^{13}C-DPPC) in combination with a treatment dose of surfactant (100 mg/kg) showed an endogenous surfactant PC pool size (before treatment) ranging from 1 to 15 mg/kg [34], while term infants without RDS have three times or more that value [35]. As synthetic rates of surfactant in preterm infants are low, it is clear that pulmonary surfactant can only be rapidly increased by surfactant replacement therapy.

61.5 Effects of Surfactant Therapy on Surfactant Kinetics in Preterm Infants with RDS

Although the administration of exogenous surfactant has become the routine treatment of RDS in the preterm infant, there is very little information regarding effects of exogenous surfactant on endogenous surfactant metabolism.

In healthy adult rabbits *in vivo*, administration of surfactant to the left lung only, resulted in increased incorporation of palmitic acid from plasma into surfactant PC in the left lung but not in the right lung [36], suggesting stimulation of endogenous synthesis.

In preterm ventilated lambs, surfactant treatment stimulated [^{3}H]palmitic acid incorporation into surfactant PC after correction for the increased surfactant pool [37]. In 3-day-old rabbits, the administration of surfactant did not, however, influence the incorporation of labeled precursor in the total lung surfactant pool [38].

In preterm infants with RDS, the half-life of PG was independent of the dose of exogenous surfactant (60 versus 120 mg/kg) [32]. This indicates that the absolute turnover had doubled after two doses of surfactant compared to one dose.

Studies in infants with stable isotopes showed that the incorporation of ^{13}C from intravenous [U-^{13}C] glucose into alveolar surfactant PC palmitate increased after exogenous surfactant treatment [26].

More recently surfactant kinetics of different doses of porcine surfactant (200 versus 100 mg/kg) were compared in preterm infants with moderate to severe RDS. This study showed that the administration of 200 mg/kg of exogenous surfactant was associated with a longer DSPC half-life and that the need of re-dosing was reduced [39]. Moreover independent risk factors for surfactant re-dosing were evaluated by multiple regression analysis in 125 RDS infants who received 100 or 200 mg/kg of exogenous surfactant. The need for surfactant re-dosing was predicted by shorter DSPC half-life, lower birth weight, worst RDS radiological score, use of conventional ventilation (*vs* high frequency) and a lower surfactant dosing [40].

In summary, surfactant kinetics is variable in infants with RDS, depending on several clinical factors. When optimizing surfactant replacement therapy and its cost-benefit ratio these clinical variables should be taken into account.

61.6 Effects of Prenatal Corticosteroids on Surfactant Synthesis in Preterm Infants with RDS

In many *in vitro* studies corticosteroids increase the activity of the enzymes involved in surfactant PC synthesis [41–45] and increase surfactant protein synthesis [43]. *In vitro* experiments with lung slices and isolated type II cells showed corticosteroids increase the incorporation of radiolabel precursors into surfactant PC, reflecting increased PC synthesis [42, 45, 46].

Prenatal corticosteroid treatment in preterm lambs rapidly increases surfactant protein mRNAs and surfactant proteins in lung tissue [47]. In a study by Kessler and colleagues [48] in premature baboons, a 72 hour treatment with prenatal dexamethasone did not increase radioactive palmitate incorporation in lung lipids, but it increased total lung phospholipids and alveolar lavage DPPC in lung lavage fluid at birth. Bunt and colleagues [49] used stable isotope labeled glucose in preterm baboons to measure the synthesis rate of surfactant PC from plasma glucose. The synthesis was doubled after a 48 hour treatment with prenatal corticosteroids. In preterm infants it was also found that two doses of prenatal corticosteroids doubled the endogenous surfactant synthesis from plasma [U-^{13}C]glucose [50].

In summary, prenatal corticosteroids enhance surfactant PC synthesis *in vivo*, but de novo synthesis rates remain low, and alveolar pool sizes are not increased within 48 h. In preterm animals with RDS, prenatal corticosteroids improve pulmonary compliance within 15 h [51] accompanied by stimulated structural development [52–57]. Therefore, the pathophysiology of the improved lung function after prenatal corticosteroids remains controversial.

61.7 Surfactant Status of Preterm Infants Recovering from RDS

The common belief that after surfactant replacement the exogenous surfactant is fully retained by the lung of the preterm infants suffering from RDS, has been recently challenged by Verlato and colleagues [31]. They studied whether reduced amounts of pulmonary surfactant contribute to post-extubation respiratory failure in preterm infants who had RDS, needed mechanical ventilation and exogenous surfactant replacement for treatment of moderate/severe RDS and could not be extubated before day 3 of life. Verlato and colleagues studied 88 preterms by the "exogenous" tracing approach. They administered endotracheally ^{13}C-DPPC as tracer before extubation, for the estimation of surfactant DSPC pool size and half-life. Patients were retrospectively divided into 3 groups, that is, a) extubation failure if, after extubation, they needed reintubation or high setting CPAP (6 or more cm H_2O of continuous positive airway pressure and a fraction of inspired oxygen grater than 0.4), b) extubation success if they did not meet the failure criteria, and c) not extubated if they needed ongoing ventilation. Sixteen, 23, and 24 neonates were categorized in the extubation failure, extubation success, and not extubated groups, respectively. Mean DSPC pool size was smaller in the extubation failure group than in the extubation success group (25 versus 43 mg/kg) and it was 37 mg/kg in the not extubated group. Mean DSPC half-life was 19, 24 and 28 hours in the extubation failure, extubation success, and not extubated groups, respectively. The authors concluded that marginal surfactant deficiency may contribute to extubation failures or need for high continuous positive airway pressure settings after extubation and perhaps more importantly draw the attention on individual differences in exogenous surfactant handling or on individual differences in endogenous synthesis. Interestingly a recent report in abstract form, showed that the administration of exogenous surfactant (Infasurf 3 mL/kg) in infants below 28 wks of gestation and beyond 7 days of age resulted in an improvement in respiratory severity score [58].

61.8 Neonatal Pneumonia

Pneumonia in children and newborn infants may be associated with surfactant dysfunction and severe acute RDS [59–64]. There are studies involving a small number of neonates who have been treated with rescue surfactant replacement for sepsis and pneumonia that have demonstrated improved gas exchange compared to no surfactant treatment [59, 61, 62, 65–67]. Verlato and colleagues [68] used the "exogenous" tracing approach with an endotracheal administration of ^{13}C labeled DPPC to study surfactant kinetics in full-terms with pneumonia, and in preterms with RDS. In this small study the authors found that the amount of DSPC recovered from the tracheal aspirates was not different among the study groups whereas its half-life was significantly shorter in full-terms with pneumonia (19.3 ± 7.3 h) than in preterms with RDS (28.7 ± 15.9 h).

Conclusion: If it is apparent from one side that large randomized controlled studies are necessary to evaluate the effects of surfactant treatment on morbidity and mortality, it may well be that the dose and time intervals of exogenous surfactant therapy ought to be different in neonatal pneumonia from the schemes currently used for neonatal RDS.

61.9 Meconium Aspiration Syndrome (MAS)

Aspiration of meconium into the lungs directly inhibits surfactant function and induces an inflammatory response in the lung with possible detrimental effects on type II cell function, and thereby surfactant metabolism. Surfactant function is inhibited by meconium in a concentration-dependent way [69, 70]. Meconium increases the minimum and maximum surface tensions and lowers the surface-spreading rate of surfactant [69–71]. Studies of surfactant concentrations and composition in MAS are scarce.

Cleary and colleagues [72] found decreased SP-A and SP-B levels in the large aggregates of surfactant in a rat model of MAS. However, phospholipid and DPPC levels did not change significantly after meconium instillation in either lung tissue or bronchoalveolar lavage fluid (BAL). Analyses of BAL fluid from 8 ventilated infants with MAS revealed no difference in phospholipid and SP-A content compared to control subjects [73]. However, concentrations of non-surfactant protein and albumin were more than 3 times those found in normal lung. In MAS infants who required ECMO, surfactant phospholipids, PC, and SP-A in tracheal aspirates increased during the ECMO treatment [74, 75]. One study investigated the surfactant kinetics in the presence of meconium [76]. This *in vitro* study in type II cells of adult rats showed that meconium in low concentrations (1%) increases the PC secretion by type II cells, but had no effect on surfactant PC synthesis. Higher meconium concentrations were toxic to cultured type II cells, though the effect of these higher concentrations on surfactant synthesis is not known.

Janssen and colleagues studied surfactant metabolism in MAS infants on ECMO with the "endogenous approach" using [U-^{13}C]glucose as a precursor for surfactant PC palmitate [23]. The FSR in MAS infants was ~3.3 %/day (compared to controls 8%/day, p = 0.058) and peak enrichment was significantly lower than in controls p = 0.027), suggesting lower synthesis in MAS. PC concentration in ELF in MAS was ~4 mg/mL (significantly lower than controls: 12.8 mg/mL). The half-life of endogenous surfactant was 69 h (not different from controls). With endotracheally administered $^{2}H_3$-DPPC as label, surfactant pool PC size was measured to be ~50 mg/kg

in neonates with MAS requiring ECMO, which was not significantly different from controls or other ECMO infants with persistent pulmonary hypertension of the newborn [77]. Exogenous administration of surfactant in animal models of MAS improves lung function and morphology, especially when surfactant is given at a high dose (200 mg/kg) [78]. Several studies showed an improvement in oxygenation after surfactant therapy, although most infants required 2 or more doses of exogenous surfactant [79]. In a randomized trial 3 doses of bovine surfactant (150 mg/kg) were administered to 20 infants with MAS. Infants treated with surfactant had improved oxygenation, a reduction in severity of pulmonary morbidity, a decrease in ECMO requirement, and a decrease in hospitalization time compared to the control group (n = 20) [80].

Lotze and colleagues [81] performed a trial in term infants with severe respiratory distress who received 4 doses of bovine surfactant (100 mg/kg/dose) at ~30 h after birth. Half of these infants had MAS. They were unable to demonstrate a difference in oxygenation, pulmonary morbidity, or hospitalization, although the need for ECMO in the surfactant-treated group was decreased. From these studies it seems that surfactant therapy in MAS is most effective when given in the early phase of respiratory failure, and at a high dose. Lavage with surfactant could remove meconium, inflammatory cells, edema fluid, proteins, and other debris from the lungs, leaving behind a layer of functional exogenous surfactant [82]. Animal studies showed a beneficial effect from surfactant lavage on pulmonary function, radiographical and histological appearance. There are few reports of lavage therapy in human neonates with MAS [83]. However, the patient groups were small and no control group was included. Recently, a multicenter, randomized controlled trial comparing surfactant lavage with standard treatment of MAS has been reported [84]. A trend towards shorter duration of ventilation and improvement in oxygenation were noted, but the differences were not statistically significant.

In conclusion, surfactant inactivation seems to play a more important role in the pathophysiology of MAS than surfactant deficiency. In the sickest MAS infants on ECMO, surfactant synthesis is disturbed. Surfactant therapy seems to be effective in MAS, and should probably be given at a high dose and at an early stage in the development of the disease.

61.10 Congenital Diaphragmatic Hernia (CDH)

Although CDH lungs are immature and morphologically show some resemblance to lungs of preterm infants with RDS, it is still unclear whether a primary surfactant deficiency is present in human CDH. Several animal models have been developed to study the pathogenesis of CDH. In bronchoalveolar lavage (BAL) fluid of surgically created CDH lambs, the amounts of phospholipids, PC, SP-A and SP-B are decreased compared to controls [85, 86]. However, the amniotic L/S ratio is not different in CDH lambs compared to control lambs [86]. *In vitro* studies in isolated type II cells of CDH lambs show a decreased incorporation of choline into PC [85], suggesting decreased surfactant synthesis in CDH. Human studies show contradictory results; the L/S ratios in amniotic fluid have been reported to be either decreased or to be normal [87]. SP-A and DSPC concentrations in amniotic fluid were lower in fetuses with CDH who died or required extracorporeal membrane oxygenation (ECMO) [88]. Autopsy studies in CDH infants who died at birth or within the first few days of life showed decreased SP-A in lung tissue [89]. No difference in L/S ratio, PC, and PG concentrations in BAL fluid from CDH infants was found compared to age-matched controls [90].

Cogo and colleagues have recently conducted a series of studies using stable isotope technology to trace pulmonary surfactant in newborn infants with CDH who were treated with mechanical ventilation and did not require ECMO. In the first study using the "endogenous approach", surfactant DSPC synthesis and metabolism were compared between CDH patients and control subjects. Secretion time was 8.3 ± 5.5 and 8.5 ± 2.5 hours and peak time 51.9 ± 15.2 and 51 ± 13 hours in infants with CDH and in control subjects, respectively. FSR was not different for infants with CDH and control subjects ($p = 0.4$). It was concluded that surfactant DSPC synthesis and kinetics were not significantly deranged in infants with CDH compared with control subjects. They also speculated that factors other than surfactant deficiency, such as lower surface area or increased DSPC catabolism, may contribute to surfactant pool alteration in CDH [22]. In a second study, Cogo and colleagues [35] studied surfactant DSPC half-life, turnover and apparent pool size in CDH newborns with no ECMO by the "exogenous tracing approach". In 13 CDH infants DSPC half-life was shorter (24 versus 53 h), turnover faster (0.6 versus 1.5 d-1), apparent pool size smaller (34 and 57 mg/kg body weight) and DSPC amount from tracheal aspirates lower (2.4 and 4.6 mg/mL Epithelial Lining Fluid [ELF]) than in controls. The data from the "exogenous tracer" suggested that surfactant kinetics was grossly abnormal in mechanically ventilated CDH. This study could not ascertain whether alterations of DSPC kinetics in CDH infants were caused by a primary surfactant deficiency or were secondary to oxygen therapy and ventilator support. In the third study of this series Cogo and colleagues [91] developed a new approach to measure DSPC net synthesis and kinetics by using dual stable isotope tracer approach i.e., combining the "endogenous" and "exogenous" techniques in the same patient. All infants simultaneously received an intratracheal (carbon-13 DPPC) and an intravenous (deuterated palmitic acid) stable isotope tracer. DSPC net synthesis from plasma palmitate was nearly identical in infants with CDH and control subjects (8.6 and 8.1 mg kg^{-1} d^{-1}). DSPC apparent pool size was 36.7 ± 7.5 and 58.5 ± 9.1 mg/kg, $p = 0.07$ and half-life was 26 and 50, $p = 0.03$ in infants with CDH and control subjects, respectively. Both DSPC turnover and percentage

of catabolism/recycling significantly correlated with duration of mechanical ventilation. In summary, this study confirmed that synthesis was not reduced in ventilator dependant CDH patients, that DSPC turnover was faster, presumably reflecting an increased DSPC catabolism/recycling, and the authors speculate that increased catabolism may ultimately lead to a secondary surfactant deficiency.

When patients with CDH were studied on ECMO by the "exogenous" tracer [77] surfactant PC pool size was comparable in CDH infants and in term newborn with severe respiratory failure who were not on ECMO (73 versus 69 mg/kg respectively). In CDH infants on ECMO Janssen [23] measured FSR by using [U-^{13}C]glucose as a precursor and found it to be lower than in controls (2.4 versus 8%/day) with a similar half-life of endogenous surfactant of ~65 h. It is unclear at present if these discrepancies in pool sizes and synthesis were due to ECMO or the severity of the patients studied. There are only a few reports of surfactant treatment in human CDH infants [92–94]. Bos and colleagues [92] showed an improvement of oxygenation in 3 of 5 infants with CDH after surfactant administration. When surfactant was given prophylactically to high-risk neonates with CDH, all 3 infants survived [93]. Surfactant treatment in 9 infants with CDH who required ECMO had no beneficial effect on lung function, morbidity, or survival [94]. Recent studies on surfactant supplementation in CDH [95] show no benefit associated with surfactant therapy for term infants with a prenatal diagnosis of isolated CDH and a lower survival rate in preterm infants with CDH [96].

Even today it is still not clear if there is a primary surfactant deficiency in CDH. We suggest that there is inactivation of surfactant function due to the intensive ventilation [97], which is often necessary in CDH infants, although, a decreased surfactant synthesis due to severe hypoplasia of the lungs (especially in the more severe cases of CDH) cannot be excluded.

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62

Respiratory Distress Syndrome: Predisposing Factors, Pathophysiology and Diagnosis

Mikko Hallman and Timo Saarela

62.1 Introduction

Respiratory distress syndrome of newborn infants (RDS), called infantile RDS or IRDS, previously idiopathic RDS or hyaline membrane disease (HMD), is the most common serious disease affecting the newborn. Since the first description of hyaline membranes by Hochheim in 1903, a number of speculations and theories flourished concerning the primary cause [1]. During the past 40 years the advances in neonatal intensive care have been based on the understanding of molecular, structural and functional lung development, the knowledge of the cardiorespiratory adaptation in premature infants, the pathophysiology of RDS, molecular biology, pharmacodynamics and technological applications. As a result of the successful marriage between clinical investigation, applied and basic sciences, the mortality of RDS has dropped from about 50% to close to 5%. The infants affected by RDS are considerably more immature since previously many immature infants died before they developed HMD.

In 1926 Van Neergard described the hysteresis, i.e., higher airway pressure requirement during inspiration of the collapsed lung than during expiration of the air filled lung that additionally retained some air when the pressure was removed altogether. The theoretical significance of this phenomenon was not appreciated until surfactant was described by Clements and Pattle in the 1950's [1]. In 1959, Avery and Mead discovered that the airways of infants dying of HMD had deficient surface activity [2]. This finding was confirmed and further tested several years later when surfactant, then assumed to be dipalmitoyl phosphatidylcholine, was nebulized to the airways of preterm infants with RDS. However, the results were disappointing [3]. It took another 20 years to further define the structure of surfactant and to show that surfactant deficiency at birth is due to lack of differentiation of the alveolar epithelium. In randomized therapeutic trials it was possible to show that natural surfactant given in established RDS or at birth decreased the severity of respiratory failure and increased survival and survival without bronchopulmonary dysplasia (BPD) [4].

In the post surfactant era the management of RDS has improved and the focus has shifted to further improvement on the long-term pulmonary and neurosensory outcome. Despite advances in the management of most immature infants shortly after birth, prolonged intensive care is often required, the outcome is often less than optimal and attempts to prevent preterm births have failed. However, antenatal glucocorticoid treatment in threatened preterm birth has decreased the risk and severity of RDS. The concerns of adverse effects of antenatal steroid have not materialized.

As a result of effective early management, infants with RDS have an early remission of respiratory failure, translating into minimum requirement of supplemental oxygen or ventilation ("new RDS"). Despite this, the risk of BPD persists among the population of extremely preterm (ELGA, <28 weeks) infants despite mild symptoms of respiratory distress after birth. The phenotype of RDS is influenced by genetic and constitutional factors, most notably by the length of gestation at birth.

62.2 Risk Factors

RDS is a disease caused by immaturity, i.e., inadequate functional differentiation of the alveolar epithelial cells at birth and acute lung injury. The clinical features are dependent on a number of constitutional and genetic features and on therapeutic practices. The risk factors have changed as a result of improved antenatal anticipation of preterm birth and pharmacologic acceleration of fetal lung maturity.

The incidence of RDS has remained rather stable. Previously the disease predominantly affected near-term and term infants. More recently the gestational age of the RDS

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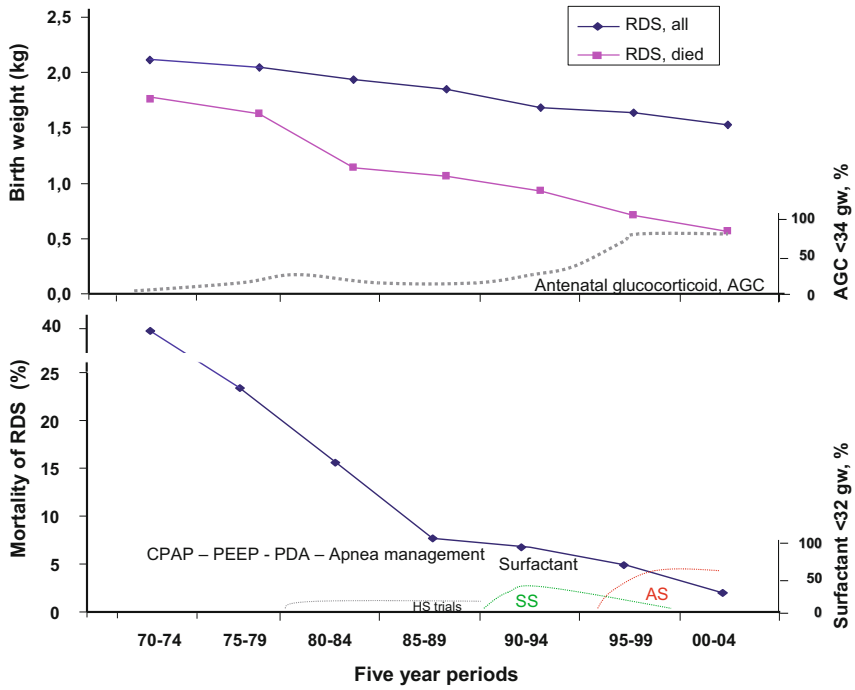

Fig. 62.1 Demographics of RDS during development of perinatal-neonatal treatment practices. Trends in mortality, and in birth weights of the infants with RDS during 1970–2004. The figures are from Finland with prematurity rate ranging from 5.2 to 6.0 percent during this period. The national incidence figures of RDS perinatal register have remained the range of 0.4–0.6% during the past 20 years, whereas the incidence figures from tertiary hospitals have ranged from 0.6 to 0.9%. Major developments in treatment practices are indicated. *HS* human surfactant; *SS* synthetic surfactant; *AS* animal surfactant. The figures are in part published, in part obtained from Hospital records and from Finnish National Institute for Health and Welfare (Dr. Mika Gissler acknowledged)

population has decreased. This has taken place because prevention of RDS in the near-term and preterm population has become partially successful, and because early treatment of newborn infants with very low gestation allows them to survive until the diagnosis of RDS is established. Fig. 62.1 shows the incidence of RDS (1970–2004) and mortality of RDS over 35 years in a homogeneous Caucasian population. The incidence of RDS among term born infants was 1 in 2000 [5]. The incidence increases by each week of decreasing the length of gestation. At 34–36 weeks it ranged from 2 to 8%, at 30–33 weeks from 10 to 35%, at 28–29 weeks from 30 to 50%, and in ELGA infants the risk exceeds 50%. High incidence in given gestation associates with the practice of elective deliveries without labor [6, 7], low use of antenatal glucocorticoid before preterm births [8] and Caucasian (*vs* African) ethnicity. Surfactant supplementation at preterm birth and continuous distending pressure ventilation from birth decreases the infant's risk of symptoms [9, 10]. Males have a higher risk than females, the difference corresponding to a mean of one week of gestation (Table 62.1). The length of gestation interacts with the risk factors. Multiple birth increases the risk of RDS in extremely preterm births and is a protective factor in near-term pregnancies (Fig. 62.1). The presenting twin has a lower risk of RDS (but higher risk of infection) than the non-presenting one [5]. Chorioamnionitis decreases the risk of RDS in ELGA births [11, 12]. Maternal diabetes and hydrops are recognized risk factors of RDS.

Table 62.1 Pregnancy-related states and complications associated with altered risk of RDS as compared to gestation controls

Accelerated maturation or control	Control or delayed maturation
Vaginal birth	Elective birth with labor
Elective birth with labour	Elective low risk birth w/o labour: late preterm or early term (37–38 wks)
Closeness to cervix in twin pregnancy (presenting)	Remote to cervix in twin pregnancy (non-presenting)
1st born premature twin ≥32 wks	Control premature singleton, ≥32 wks, twin pair
Singleton <28 wks	Twin <28 wks
Twin 32–36 wks	Singleton 32–36 wks
Preeclampsia 29–32 wks	Control 29–32 wks
Control <28 wks	Preeclampsia <28 wks
Chorioamnionitis 24–29 wks*	Control 24–29 wks
Gestation control	Maternal diabetes
Gestation control	RH immunization, hydrops
Circumvallatae placenta	Gestation control

* Mostly histologic chorioamnionitis. Clinical chorioamnionitis, when associated with foetal infection, is associated with respiratory distress mimicking RDS.

62.2.1 Genetic Factors Influencing Susceptibility to RDS

According to a twin study comparing the concordance of RDS between monozygotic and dizygotic same sex twins,

the genetic contribution to the susceptibility to RDS is only 0–40%. However, many genetic effects are due to gene-gene or gene-environment interactions. They undermine the estimates of the genetic effects on the basis of twin studies. For instance, the presenting twin has a lower risk of RDS compared to the non-presenting one and this difference is due to gene-environment interaction [13, 14].

Many surfactant lipids, surfactant proteins and proteins involved in intracellular processing of surfactant phospholipids are essential in respiratory function. Mutations that disrupt surfactant function include SP-B, ABCA3, and sometimes SP-C cause fatal lung disease that initially resembles RDS [15] (see Chapters 60 and 61). Several other proteins, including SP-A and SP-D have important, partly redundant functions that influence the susceptibility to infections and inflammatory insults. Genes with identified roles in lung function may have mutations or common allelic variants that influence surfactant function directly or indirectly. Studies thus far show that some genes expressed in type 2 cells influence the individual risk of RDS, including polymorphism of SP-A, SP-B, SP-C and ABCA3 [14]. RDS in near-term infants appears to have a different genetic background compared to RDS in very preterm infants. Many likely candidate genes remain to be studied. RDS is tightly linked to the degree of prematurity. The mediators influencing the risk of spontaneous premature birth and the risk of RDS are in part intertwined [16]. Typical multifunctional genes include collectins SP-A and SP-D that may influence cytokine responses mediating surfactant synthesis and the preterm labor process.

62.3 Symptoms and Clinical Findings

62.3.1 Lung Function

RDS is characterized by early rapid progression of respiratory distress that leads to respiratory failure in the absence of treatment within 1–48 hours. The healing phase starts within 1–6 days and is generally more gradual than the early phase. The clinical manifestation differs remarkably on the basis of the length of gestation as a result of constitutional differences in lung structure and host defense functions. The unifying features in RDS are the transient deficiency of surfactant in epithelial lining and the early lung injury. Very low functional residual capacity and low dynamic compliance are typical findings. Short inspiratory time constant have been reported early in the course of RDS, whereas during later in the course it may increase as a sign of airway obstruction [17]. Modest abnormalities in lung function may persist even weeks after the recovery from uncomplicated RDS in near-term infants.

The post-hospitalization course of RDS infants without developing BPD reveal a higher incidence of wheezing during infections and a higher hospitalization rates due to respiratory symptoms in infancy. These features persist during the infancy regardless of the gestation at birth, suggesting residual lung injury or aberrant immune activation during infection [18, 19].

62.3.2 Typical Course of RDS

Half of the infants with RDS are born between 29 and 33 weeks of gestation. First symptoms are observed very soon after birth. Tachypnea, nasal flaring, subcostal and intercostal retractions, cyanosis and expiratory grunt are characteristic, although not very specific. In RDS, the symptoms continue longer than 24 hours, unless specific therapies are applied. Cyanosis due to right-to-left pulmonary shunting of the blood is evaluated by oxygen saturation monitoring and objectively documented by measuring the arterial blood gases and calculating the alveolar–arterial O_2 tension difference. In the early phase, the breath sounds are often diminished; later inspiratory stridor may be evident. Observing the chest movements and the symmetry of the breath sounds is indicated, as pneumothorax sometimes complicates the course even with current advanced treatment strategies.

The early progressive phase of RDS is abrogated from most infants receiving early surfactant treatment. As a result of mild symptoms the diagnosis remains uncertain, unless pretreatment surfactant diagnostics are available or the patient had a favorable response to exogenous surfactant. The surfactant-induced remission may be followed by either recovery or relapse that requires retreatment. The response to exogenous surfactant is less predictable and generally less striking if surfactant is given later (>6 hours after birth). These infants have mostly uncomplicated recovery. Some develop patent ductus arteriosus (PDA). Apnea, feeding intolerance and infection are other complications.

62.3.3 Extremely Preterm Infants

Infants born before 28 weeks of gestation have simplified saccular airways, no true alveoli and wide interstitial spaces. The highly compliant chest cage generates striking retractions despite apparently deficient transpleural pressures. The surfactant pool is generally low and surfactant is susceptible to inhibitors. Without treatment the infants often die before development of HMD. The aim of pulmonary treatment starting at birth is by using gentle ventilatory treatment to limit the emerging lung injury and provide continuous distending pressures. Prophylactic surfactant is often the treatment of choice. Surfactant frequently induces a sustained remission characterized by requirement of generally small quantities if any supplemental O_2. Relapse of respiratory failure is common, and therefore retreatment is given early. The course of RDS is characterized by a high incidence of cardiopulmonary complications. Hypoxic respiratory failure, respiratory distress

associated with asphyxia, low cardiac output and hypotension, early symptomatic PDA, air leak syndromes (pneumothorax, interstitial emphysema) and pulmonary hemorrhage complicate the course. Severe RDS, particularly its life-threatening hemodynamic complications and associated infections are strong risk factors of extrapulmonary diseases (intraventicular hemorrhage; IVH, necrotizing enterocolitis; NEC, retinopathy of prematurity) and of BPD.

Despite antenatal steroids, early surfactant, noninvasive ventilatory treatment practices and other adjunct treatments, such as late cord clamping, dopamine, dobutamine, caffeine, and inhaled nitric oxide or corticosteroid to special high risk groups, even very mild or absent RDS, a significant risk of BPD remains [20]. The degree of prematurity in ELGA infants is a strong risk factor for BPD.

62.3.4 Near-Term or Term infants

Near-term or term infants with RDS are often born with elective cesarean section without active labor. Other risk factors include acute asphyxia, maternal diabetes, rapid progress of labor and delivery, and genetic risk [21]. Infection or severe asphyxia may cause a syndrome mimicking RDS but actually representing acute RDS due to primary alveolar injury (ARDS). Constitutional features alter the manifestation of RDS. The breathing is rapid but apparently effortless as the solid chest cage does not clearly reveal retractions. Undermining the early symptoms escalates the later respiratory course. Sometimes persistence of pulmonary hypertension, hyperexpansion of the lung fields or unexpected pneumothorax are dominant features. Studies reveal surfactant insufficiency despite atypical presentation and most patients respond to high distending pressures and to a high surfactant dose.

In near-term RDS, the structural development of the respiratory system explains the phenotype. The small size of newly developed true alveoli predisposes to therapy resistant atelectasis. The stable chest cage and strong respiratory drive generates very high transpulmonary pressures during inspiration, improving the clearance of edema fluid and contributing to the risk of pneumothorax. The pulmonary vasculature has a prominent smooth muscular layer that extends distally, and contracts in hypoxia and acidosis.

The clinical recovery from severe RDS often takes place within a short period. Continuing severe respiratory symptoms beyond the early neonatal period raises the possibility of underlying disease, such as hereditary interstitial lung disease (congenital alveolar proteinosis).

62.4 Diagnostics

The diagnosis is based on a combination of characteristic symptoms, clinical findings and the chest X-ray. Typical chest X-ray features reveal a diffuse reticulogranular pattern with superimposed air bronchograms (Fig. 62.2), indicating atelectasis and edema of respiratory units, distal to respiratory bronchioles. The prominent air bronchograms representing aerated bronchi and larger bronchioles become distinct when they are superimposed on a background of atelectatic lung parenchyma. Normal newborns occasionally have air bronchogram overlying left cardiac silhouette. The severity of RDS on the basis of the chest X-ray has been graded [22, 23]. Pulmonary whiteout (grade 4) is generally a sign of a severe disease; it is also observed in mild disease very soon after birth (<1h) or soon after extubation. Pulmonary edema is a prominent chest X-ray finding in RDS complicated by PDA. In near-term RDS population, reticulogranularity tends to be less distinct and the lung fields reveal a ground glass appearance. Some infants with rather advanced gestation have hyperexpanded lung fields and pulmonary hypertension. This entity has been called type 2 RDS [24]. Distinct chest X-ray may be explained on the basis of relatively large diameter alveolar ducts that dilate during high transpulmonary pressures generated during inspiration in near-term infants. The anatomical disproportion between small newly developed true alveoli and large alveolar ducts may explain the discrepancy between the chest X-ray and abnormal gas exchange.

Surfactant analyses performed on gastric or airway aspirates at birth differentiate RDS with specificity of and sensitivity of 50–75% and 78–97%, respectively, depending on the method of surfactant analysis and site of specimen [25, 26]. The false positive prediction may be due to blood contamination, and an occasional false negative prediction due to surfactant inhibition as a precipitating cause of RDS.

62.4.1 Differential Diagnosis

Transient tachypnea (wet lung) becomes distinguishable from RDS within several hours after birth. With new treatments these two diseases may be difficult to distinguish. Bacterial pneumonia, most notably due to Group B *Streptococcus* may even coincide with RDS; pleural exudate is common and focal infiltrates uncommon in infection. Congenital chylothorax is distinguished by typical appearance of pleural exudate. Severe lung edema due to secondary causes mimics RDS. Rare cases of small preterm infants with minimal respiratory symptoms develop cystic interstitial emphysema soon after birth (Wilson-Mikity syndrome) [27], a distinct form of obstructive inflammatory lung disease.

Congenital cardiac diseases associated with severe respiratory distress, most notably anomalous pulmonary venous return with abnormal infra diaphragmatic pulmonary vein return (Scimitar syndrome) are to be distinguished promptly from RDS as early diagnosis is required for proper treatment. Early respiratory distress due to diaphragmatic hernia, other causes of lung hypoplasia, esophagus atresia, isolated fistula,

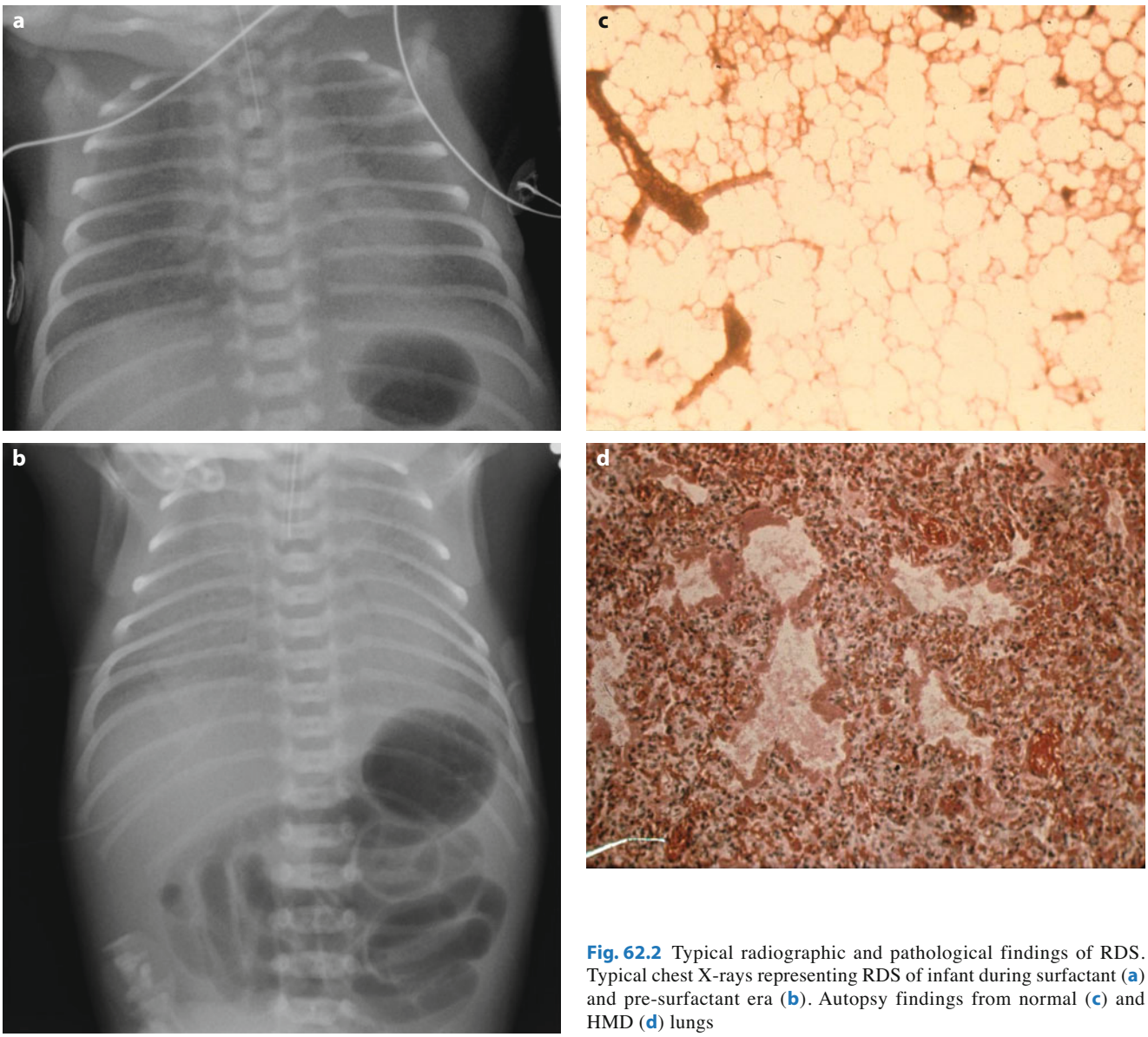

Fig. 62.2 Typical radiographic and pathological findings of RDS. Typical chest X-rays representing RDS of infant during surfactant (**a**) and pre-surfactant era (**b**). Autopsy findings from normal (**c**) and HMD (**d**) lungs

cystic adenomatous malformation, sequestration and tumors require early diagnosis.

A slow recovery from RDS may be a genetically determined constitutional feature, frequently due to PDA. Lack of recovery is a feature of PAP that often has positive family history [15]. Persistence of pulmonary hypertension (PPHN) mostly develops as a consequence of primary lung disease, often RDS. Alveolar capillary dysplasia is suspected on the basis of persistence of severe, persistent PPHN; pathologic descriptive diagnosis reveals severe growth disturbance of the lung and dysmorphic alveolar-capillary tissue [28]. In very severe lung disease, it is prudent to recover DNA, an airway specimen, and if possible to process lung for pathologic exam.

ARDS may sometimes develop in severe infection. These patients mostly respond acutely to a high dose of exogenous surfactant. However, the therapy has not significantly improved the outcome of this severe, heterogeneous syndrome.

62.5 Predisposing Factors

RDS is characterized by lung immaturity that results in poor cardiorespiratory adaptation and to respiratory failure without treatment. Surfactant deficiency is the primary cause of the tendency to generalized atelectasis. The peripheral lining of air spaces at 22 weeks of gestation contains some epithelial cells that morphologically resemble type 2 cells. Later in gestation the number of morphologically differentiated type 2 cells increase until the term, when the alveolar epithelium is

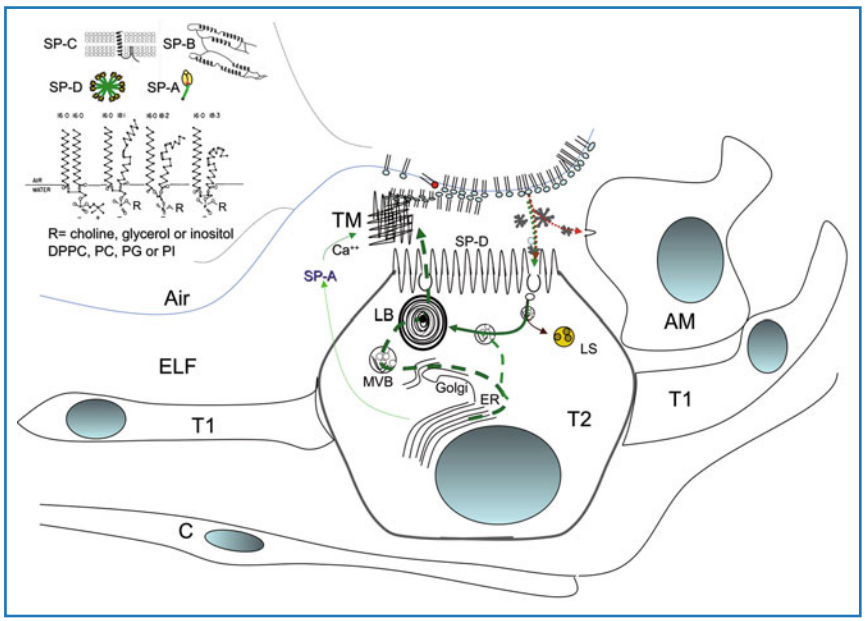

Fig. 62.3 Surfactant metabolism in alveolar epithelial cells and in epithelial lining fluid (ELF). Surfactant components are synthesized endoplasmic reticulum (ER) of type 2 cells (T2). Most surfactant components (phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, SP-B and SP-C) are processed [via Golgi apparatus and multivesicular bodies (MVB)] into lamellar bodies (LB). After secretion into alveoli, surfactant incorporates Ca^{++}-SP-A/SP-D, and transforms to tubular myelin (TM) that has rapid surface adsorption. During tidal breathing movements, some surfactant shuttles between surface and ELF. Eventually it is inactivated and taken up by alveolar macrophages (AM) and T2 cells for catabolism in lysosomes (LS) or for reprocessing into LB (recycling). In RDS there are multiple surfactant defects that are augmented by airway- and alveolar injury

loaded with type 2 cells. The rate of differentiation of the surfactant system and other pulmonary host defense systems each undergo a distinct antenatal development.

The major surfactant component, dipalmitoyl phosphatidylcholine (DPPC) concentrates at the air liquid interphase as a tightly packed lipid film (Fig. 62.3). However, it adsorbs very slowly from subphase to surface. Other surfactant components provide the extraordinary rapid surface adsorption of DPPC. These compounds are mostly unsaturated phospholipids containing anionic charge (phosphatidylglycerol, phosphatidylinositol) and hydorophobic surfactant proteins (SP-B and SP-C), containing stretches of entirely hydrophobic amino acids interrupted by individual amino acids with cationic charge. Besides deficient quantity and quality of surfactant in the epithelial lining of alveoli, the surfactant system after birth is a target of insults that deteriorate its function (inhibition, inactivation, degradation, de-aggregation, displacement) as part of the manifestation of lung injury. Despite characteristic lung injury, RDS is a self-healing disease, as the activation of the inflammation and anti-inflammatory endocrine axis promotes spontaneous maturation of the surfactant system and other host defense systems of the lung. This presentation focuses on alveolar epithelial cells.

62.5.1 Surfactant System

The lung has developed relatively late in evolution (~250 million years) and human evolution is extremely recent (~5 million years). The control of gene expression generates much of the diversity in human genome. Surfactant in healthy postnatal lung serves as a multifunctional, dynamic lipid protein complex. The lipophilic components, phospholipids, pro-SP-B and pro-SP-C, are synthesized in the endoplasmic reticulum, followed by serial cleavage of proproteins and transport via the Golgi apparatus to multivesicular bodies that transform to intracellular lamellar bodies. Shortly after secretion from lamellar bodies into the alveolar lining, surfactant binds Ca^{++} and SP-A and transforms to tubular myelin surfactant that has a very rapid surface adsorption. At the air-liquid interphase surfactant serves as a highly dynamic lipid layer that greatly reduces surface tension. Surfactant additionally serves as a lubricant that reduces barotrauma and, perhaps most important, serves as an immunomodulator that influences a range of pulmonary functions involved in defense against microbes and other insults regardless of etiology.

In the alveolar lining a minor fraction is removed by airway cilia. Most of it dissociates into smaller, protein-poor aggregates that are taken up by both type 2 alveolar cells and alveolar macrophages. In type 2 cells this surfactant may be reutilized and reincorporated into lamellar bodies (Fig. 62.3). Some genes are preferentially, but not exclusively, encoded in the lung, including SP-A, SP-B, SP-C and SP-D, and ABCA-3 is involved in intracellular processing of surfactant complex. The expression of these and many other proteins, including a number of signaling and transcription factors, hormones, growth factors and their receptors control the structural lung development and lung surfactant homeostasis. In human species the prematurity rate is exceptionally common. The high-risk fetuses are exposed to various stresses that induce abnormal fetal lung maturity.

Deletion of genes encoding SP-B, ABCA-3, a mutation in transcription factors TTF-1, C/EBPα, FOXA2, a glucocorticoid receptor GRα and some other genes selectively delay lung development causing fatal murine respiratory distress at birth. Some other genes are less selective and lack of them

may disrupt the development very early in gestation or delay the differentiation [29]. Biochemical maturation of the surfactant system involves increase in the activity of the enzymes required for synthesis of surfactant phospholipids. Surfactant proteins SP-A and SP-B show a distinct increase in the expression during near-term development, whereas the antenatal differentiation of SP-C and SP-D is somewhat different. The increase in intracellular synthesis of surfactant leads to intracellular processing and secretion of the complex that eventually accumulates into amniotic fluid [30]. Glucocorticoid is the main hormone regulating spontaneous functional differentiation of the lung [31]. The effects of glucocorticoid on the surfactant system are mediated by several pathways, one being fibroblast-derived growth factor that influences the differentiation of type 2 alveolar epithelial cells involved in surfactant synthesis and secretion. Glucocorticoid typically promotes differentiation of other functions in lung and in other organs, and structural maturation of the lung. High glucocorticoid activity decreases the growth. Absence of glucocorticoid also leads to lung hypoplasia and respiratory failure at birth. Other hormones contributing to biochemical lung maturity include adrenergic agents, thyroid hormone and prolactin, whereas testosterone delays lung maturity. Several growth factors and cytokines also influence the differentiation and growth of alveolar tissue.

Besides the transcription factors and signaling involved in normal differentiation of the alveolar epithelial cells, salvage pathways, influencing the lung beyond the timetable of normal differentiation operate in intrauterine stresses. Proinflammatory cytokines signal both the acceleration of lung maturation and spontaneous preterm labor in chorioamnionitis. IL-1 has been identified as a primary agonist upregulating the surfactant system in intrauterine inflammation [32]. In experimental settings an acute intra-amniotic infection rapidly induces surfactant maturity within 1–2 days. Endotoxin or proinflammatory cytokines introduced to the amniotic fluid have apparently little acute effect on fetal well-being as they accelerate differentiation of the surfactant and induce lung inflammation that may extend systemically. In contrast, severe systemic infections and inflammatory response syndrome have adverse fetal consequences [33, 34].

In hypoxia, expression of hypoxia inducible factors (HIF-1α, HIF-2α) is activated. HIFs are oxygen-regulated transcription factors that control the expression angiogenic genes, including VEGF. HIF-1α and other genes are involved in the development and growth of the capillary network around the airspaces. HIF-1α is required for differentiation of fetal type 2 alveolar cells. Increase in VEGF stimulates surfactant synthesis [35]. Extremely severe hypoxemia and restriction of placental supply of nutrients deteriorates fetal well-being and retards lung growth. These very preterm infants are prone to postnatal infections and chronic lung disease.

Deficient synthesis, intracellular processing and secretion of surfactant cause high surface tension in immature lung. Each of these steps may be rate limiting in RDS. In infants developing RDS, generally low although variable quantities of surfactant is present in the airways at birth. As a result of premature birth, a number of mediators that advance the epithelial differentiation, including glucocorticoid, secretion of adrenergic agent and of proinflammatory cytokines, promotes surfactant synthesis and secretion is activated. Surfactant sufficiency is established within 2–7 days in most cases [36–38].

62.5.2 Secretion of Fetal Lung Liquid

During the second trimester, the rapidly growing fetal lung already secretes Cl^- (150 mEq/L) [39]. During the second trimester this results in osmosis-driven liquid secretion of 0.3–0.8 liter per 24 hours of protein-poor liquid. At the same time, 7–10% of the cardiac output perfuses the alveolar tissue. A normal fetus makes periodic breathing movements with a small tidal volume (0.2–3 mL), resulting in transfer of liquid in and out from the airways. The fetus swallows some of the lung liquid, although remarkable quantities enter the amniotic fluid, contributing 5–15% to its volume. During the second trimester most of the amniotic fluid is formed by fetal urination and cleared by fetal swallowing, with a turnover time of ~48 hours.

The secretion of the lung liquid, tidal respiratory movements and airway contractility dilate the future peripheral airways. The tidal movements of the chest wall deliver some of the amniotic fluid content to the airways [40]. In chorioamnionitis, the content of amniotic fluid in the airways activates the host defense system [32, 34]. Experimental ligation of the fetal airways results in expansion of airways and hypercellularity of lung parenchyma. Lack of fetal breathing movements leads to poor lung growth that is particularly severe when the chest (lack of amniotic fluid) or lungs (diaphragmatic hernia, intrathoracic tumor, small chest cage) are compressed. Severe oligohydramnios from midpregnancy results in lung hypoplasia and deformations, originally described as a result of fetal anuria (Potter syndrome), but also caused by prolonged rupture of fetal membranes.

Chloride ion exits the epithelial cells of the airways and alveoli through apical anion-selective channels, including the cystic fibrosis transmembrane regulator protein. The driving force of the pump is linked to the Na,K-ATPases in the basolateral membrane, involved in active transport of Na^+ into the cells, generating the electrical potential difference allowing basolateral entry of Cl^- [41, 42] (Fig. 62.4).

62.5.3 Ion Channels and Pumps Responsible for the Absorptive State of Lung

Towards term the secretion of lung liquid decreases dramatically as the lung switches from a liquid secreting to a liquid

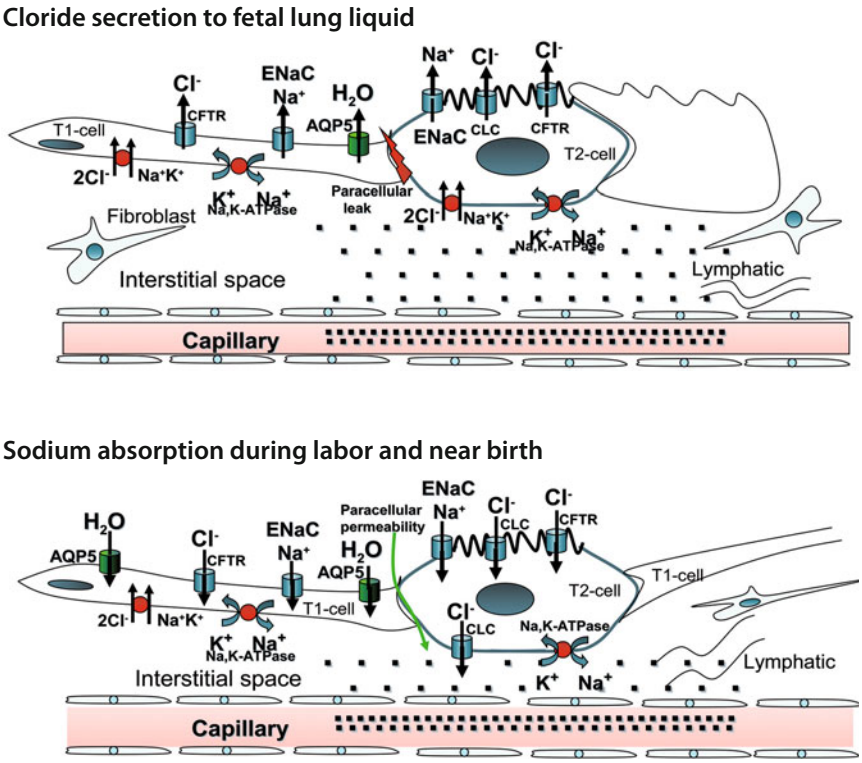

Fig. 62.4 Lung liquid secretion in fetal lung (*above*) and liquid absorption during labor and near birth (*below*) [43]. Liquid secretion is a risk factor of RDS and liquid absorption is a protective factor. Both are energy dependent processes driven by Na^+,K^+-transporters in the basolateral membrane of type 1 (T1) and T2 alveolar cells. The induction of the absorptive state involves glucocorticoid and adrenergic agents. The rate limiting activities for absorption include sodium channels (ENaC), aquaporins (AQP5 in T1-cells) and Na^+,K^+-ATPase. The fetal state of liquid secretion is driven by Na^+,K^+-$2Cl^-$ co-transporter. To maintain electroneutrality, Cl^- ion exits the apical membrane through cystic fibrosis transmembrane conductance regulator (CFTR) and through volume- and voltage- gated chloride channels (CLC). Sodium and water follow through specific channels. The resulting lung liquid promotes fetal lung growth but also increases the risk of lung damage and large paracellular leak during first breaths after birth

absorbing organ. The lung liquid during active term labor decreases by 40–50% as a result of active absorption of lung water. Lung extracellular water further decreases by another 40% during the first 6 hours of life at term.

Both Type 2 and Type 1 epithelial cells contain amiloride-inhibited ENaC protein complex (α, β, γ), attached in the apical plasma membrane. Besides the luminal transporters, basolateral Na,K ATPase and voltage gated Cl^- channels transport Na^+ and Cl^- ions to lung interstitium. Aquaporins facilitate the movement of water across cell membranes.

Adrenaline via cyclic AMP acutely induces the absorptive state of the fetal lung. Additive with adrenaline, corticosteroid decreases lung liquid formation. Adrenaline activates ENaC and corticosteroid increases the synthesis of ENaC protein, particularly the rate-limiting ENaC α. Flucocorticoid increases as a result of activation of the pituitary-adrenal axis towards normal pregnancy. In addition, adrenalin increases during labor, and a further increase takes place shortly after birth [43].

In infants developing RDS the ion channels responsible for clearance of the lung liquid are not active. This immaturity of the alveolar epithelium is likely to aggravate the symptoms of respiratory distress and activation of ion channels involved in liquid adsorption associated with the recovery. According to the favored hypothesis, wet lung syndrome is due to insufficient clearance of lung liquid and critical deficiency of the active clearance mechanism [44, 45].

62.5.4 Structural Lung Development

There are gestation-dependent changes in lung structure, including differences in microanatomy and in the amount and composition of the extracellular matrix. These factors influence the phenotype of RDS.

During the canalicular stage between 16th and 25th weeks of gestation the lung transforms from previable to a potentially viable organ, capable of gas exchange. This is accomplished by the growth and differentiation of the distal ends of the preacinar terminal bronchioles that grow and branch to two to four respiratory bronchioles. The potential gas exchange surface enlarges as a result of simultaneous vascular sprouting and acinar growth. In addition future airways and capillaries approach each other, decreasing the potential diffusion pathway of gas exchange. The epithelium undergoes thinning from columnar to cuboidal form as type 2 cells differentiate.

During the saccular stage between 23 and 34 weeks, clusters of thin-walled saccules form the acinus that simultaneously expands and grows in length as the respiratory bronchioles divide into 6–7 generations by branching. The capillaries still form a double capillary network between individual saccules. Elastic fibers are deposited along the airways and capillaries.

Towards the end of gestation and particularly after birth the elongated type 1 alveolar cells expand and increasingly cover the alveolar lining, whereas surfactant-secreting type 2

cells arrange around the alveolar corners. The alveolar stage involves microvascular maturation that starts already near term and continues during the first years. True alveoli grow from the walls of terminal saccules as spherical structures. They grow in size and the cellular intersaccular structure containing a double capillary network transforms into a single capillary network in close apposition to alveoli [46, 47].

62.6 Pathogenesis

Since the primary importance that lack of surfactant function as a triggering factor has been recognized and early surfactant therapy has been adopted, the severity of RDS has decreased considerably. Although the effect of surfactant is often dramatic in reducing or even abolishing the symptoms of respiratory distress, in some cases its effect is either transient or in rare cases missing altogether. This emphasizes the dynamic and interactive nature of additional factors influencing the alveolar stability, pulmonary circulation and structural integrity, essential to the gas exchange function of the lung. The interactive factors include the conducting airways that are intimately involved in lung injury, characteristics of the airways and cardiac function that is influenced by ductus arteriosus that tends to remain open despite improvement in alveolar gas exchange. Regardless of length of gestation and pregnancy complications, diffuse atelectasis, lung edema and hyaline membranes are a consequence of functional immaturity of alveolar epithelium. Infections and severe asphyxia and other adverse events complicate RDS and sometimes these acquired factors contribute to primary cause. Fig. 62.5. illustrates factors involved in pathogenesis that are additionally explained below.

62.6.1 Biotrauma

In addition to the pathogenic factors listed below, the innate immune system is of principal importance. The inflammatory reaction as a result of biotrauma induces both healing and tissue destruction (oxidants, lytic proteins). The balance between these numerous activities depends on management practices, constitution and hereditary factors.

62.6.2 Surfactant

High surface tension retracts the air spaces with very small principal radii (R_1 and R_2) according to the law of Laplace:

$$P \text{ (collapsing pressure)} = $$

$$= \text{surface tension constant } (\gamma) \times \left(\frac{1}{R_1} + \frac{1}{R_2} \right)$$

In true alveoli or at the tip of respiratory ducts of immature lung, the principal radii are equal ($R_1 = R_2$; $P = 2 \times \gamma/R$), whereas in tubular surfaces, the second radius is indefinite ($1/R_2 \sim 0$; $P = \gamma/R$). Surface tension is measured *in vitro* using modified Wilhelmy balance, pulsating bubble surfactometer or captive bubble surfactometer. In situ measurements during mechanical ventilation are based on microscopic observation of the alveolar surface containing droplets of specific fluorocarbons that have a constant, generally low surface tension.

Normal alveolar surface liquid lining after birth is virtually free from surface forces (γ close to 0 mN/m) *in vivo*. The rapid shallow breathing movements and the pressure waves induced by the cardiac cycle barely reshape the peripheral air spaces sufficiently to alter the low surface tension. During

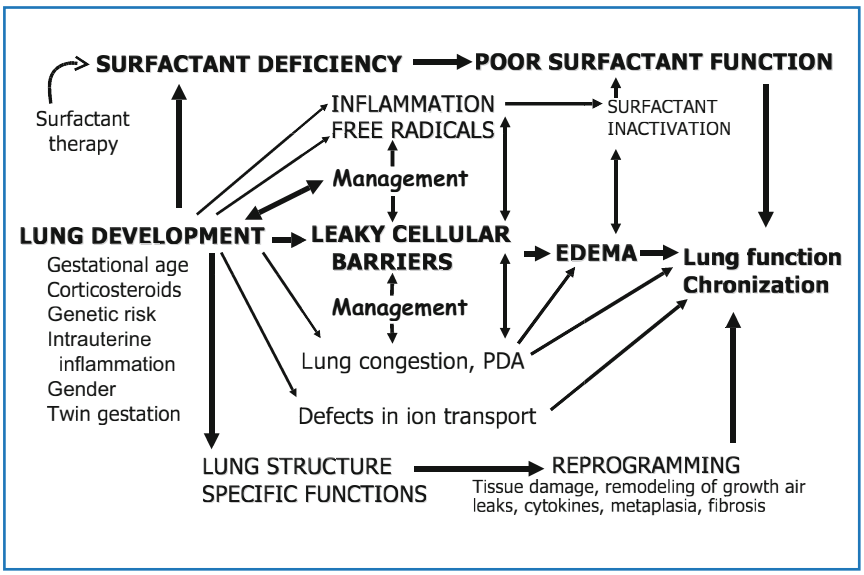

Fig. 62.5 Factors involved in pathogenesis of RDS. The primary factor is surfactant deficiency that is augmented by other defects of differentiation (e.g. lack of absorptive ion transport) and acquired factors (e.g. asphyxia, volutrauma). The resulting lung injury and inflammation promotes high permeability lung edema. Chronization of lung injury is opposed by the process of lung healing, plasticity and differentiation (e.g. induction/increase in surfactant), induced by the stress of perinatal transition

deep inspiration some increase in surface tension takes place as the surface area is significantly expanding followed by a drop in expiration (i.e., hysteresis). Due to exceptionally fast surface adsorption, the surface tension is low even during deep inspiration (10–20 mN/m) [48].

During the first breaths, lung liquid surfactant rapidly adsorbs and concentrates on the emerging air liquid interface. Lowering the surface tension from near 72 mN/m (isotonic saline in water) towards 0 mN/m, has a large impact on the forces required for liquid clearance from narrow tubular structures and alveolar saccules. Despite low surface tension, a large force is consumed against viscous resistance as the water moves out from the airways and alveoli. Transient negative interstitial pressures generated during the first forceful inspiration (as high as 50–70 cmH_2O) and positive pressures during the first cry (30–50 cmH_2O) may be exceptionally high. These pressures may cause some injury, particularly in structurally immature lung. Both large and smaller airways are exposed. Low surface tension facilitates clearance of lung liquid and protects the lung from injury. It reduces the very high pressures required to advance the meniscus towards smaller airways and alveolar saccules.

In RDS the minimum surface tension of the proteinous airway lining has minimum surface tension in the range of 20–50 mN/m. Distending pressures that maintain patency of alveoli with a radius of 50 μm and surface tension of 20–50 mN/m (similar surface tensions are evident in airway specimens recovered in RDS) is 8–20cm H_2O. As a result of slow surface adsorption surface tension increases during inspiration. High surface tension increases atelectasis (atelectotrauma) and alveolar edema and also airway resistance as it decreases the clearance of lung fluid and particularly the maintenance of alveolar patency during expiration. Surfactant deficiency additionally contributes to barotrauma (lack of lubricant property of surfactant) and volutrauma (overstretching of few remaining patent air spaces), manifesting as bronchial/bronchiolar permeability increase, perivascular edema, bronchiolar obstruction, and biotrauma.

At term birth total pool size of surfactant per kg BW is higher than in the adult (50–120 mg/kg versus 0–40 mg/kg). During the course of RDS, the surfactant system spontaneously expands and the pool size reaches the levels measured in the preterm controls by the age of 2–7 days [36]. In addition, the differentiating surfactant system is exposed to multiple injuries, delaying the recovery [38]. Besides the surface tension-reducing capacity, the individual components of the complex form an integral part of the immune defense of the lung. The coincidence of pneumonia and RDS reflects the immaturity of the innate immune system.

Besides the pool size of surfactant that critically influences the concentration of surfactant in the epithelial lining fluid, multiple factors perturb the capacity of surfactant to lower the surface tension after birth. The leakage of proteinous liquid across the epithelial lining not only dilutes surfactant but additionally reduces its ability to decrease the surface tension [36–38]. A number of proteins, cationic amino acids, carbohydrates and lipids inhibit surfactant function *in vitro*. Fibrin monomer is one of the most potent surfactant inhibitors described. Other mechanisms of surfactant inactivation include excess of proteolytic and phospholipase activities. Finally, oxidant injury by hydroxyl radicals and peroxynitrite deteriorate surfactant function [39]. Most of these noxious agents originate from inflammatory cells as a consequence of lung injury by barotrauma, hyperoxia and endotoxins.

In severe lung damage, as in some forms of interstitial lung disease, the surfactant complex may be displaced to the interstitial space. Another potential mechanism of apparently fast clearance of surfactant from air spaces is trapping within the airways that remain atelectatic or obstructed. As a result of recruitment of peripheral airways and alveoli, the trapped surfactant may reappear in airway specimens.

62.6.3 Lung Edema

Prenatal events influence the lung liquid adsorption and the amount of lung liquid at birth [40]. Spontaneous labor, advanced gestation and vaginal birth additively decrease the liquid in the airways at birth. Besides the electrochemical transport of lung liquid, water and ions move through interepithelial pores as the hydraulic conductivity and small solute permeability of the lung epithelium increases [41]. In healthy human infants the clearance of most airway liquid to lung interstitium takes place within seconds to minutes after the first breaths. Active, energy-requiring and passive mechanisms are likely involved. Both mechanisms are stretch-activated, with prominence of the energy requiring cation transport. The clearance of liquid from lung interstitium to lung circulation is more gradual, taking place during the first day as the solute directly enters the circulation or is cleared via lymphatics. The bulk of the liquid removed from the airways accumulates in distensible perivascular spaces of large lung vessels and airways, away from the gas exchange path.

Lung immaturity adversely affects the lung liquid clearance as the compliant chest cage complicates generation of high interstitial pressures and the activity of ion transport across the epithelium towards lung interstitium is low. Low ENaC expression in luminal cell membranes retards the active transport of Na^+ and oncotic transport of water to interstitial space. Term-born human infants with ENaC deficiency apparently do not develop respiratory distress as the lung liquid in term infants may have other clearance routes. During the third trimester, particularly during labor ENaC increases as a result of increased glucocrticoid and adrenergic activity. The increased risk of acute respiratory distress in near-term and even term cesarean sections without labor may be in part due to the high volume of lung liquid as a result of intranatal and early neonatal failure of activation of ion transport, including ENaC and active Na^+K^+ transport [42–44].

The adsorptive state of fetal lung liquid leads to retention and accumulation of surfactant in the airways and in air saccules, providing the critical pool of surfactant for reducing the surface tension at birth. At the same time surfactant secretion is modestly increased and continues shortly after birth. However, no rapid burst of surfactant secretion is associated with birth as originally described [46].

62.6.4 High-Permeability Lung Edema, Acute Lung Injury

The injury of alveolar and airways epithelial cells as well as capillary endothelium is a characteristic feature in RDS. The inducing events include oxygen toxicity, volutrauma/barotraumas, atelectotrauma and biotrauma (endotoxins, cytokines, infection). Regardless of etiology, the central feature is an increase in permeability to macromolecules. In RDS a profound increase in permeability exceeds the capacity of pulmonary lymphatics, resulting in interstitial edema. Airways and bronchioles are susceptible to volutrauma- or barotrauma-induced high permeability and edema-induced obstruction of the bronchioles. This enhances uneven aeration of alveolar ducts and saccules as some of them become overdistended while many are atelectatic as a result of compression by overdistended airways and of surfactant deficiency. "Effective" ventilation may not be helpful as it may increase volutrauma.

Increased epithelial permeability and high surface tension leads to alveolar edema and accumulation of surfactant inhibitors. Accumulation of fibrinogen into alveolar spaces precedes the formation of hyaline membranes. Epithelial debris, along with residue of inactivated surfactant components, is incorporated into the matrix of fibrin-rich hyaline membranes. Hyaline membranes stain with alveolar-epithelial cell-derived tissue factor that may initiate intra-alveolar coagulation [49] and they are eventually cleared by phagocytosis and fibrinolytic activity.

62.6.5 Pulmonary Hemorrhage

Pulmonary hemorrhage is a rare complication of RDS mostly in extremely preterm infants. In may be seen as an extreme form of high permeability lung edema that is associated with PDA, decrease in pulmonary vascular resistance and rapidly developing left cardiac failure and may be associated with coagulation disorder. Hemorrhage may be precipitated by surfactant therapy and inadequate PEEP (positive end-expiratory pressure) levels, allowing flux of blood from patent ductus arteriosus and rapid development of hydrostatic congestion, rupture of capillaries and airway epithelium. Treatment includes exogenous surfactant, high distending pressures and coagulation factors (pulmonary tamponade).

62.6.6 Abnormalities in Lung Perfusion

Both the epithelial differentiation and the vascular sprouting around the distal epithelium are essential for successful gas exchange. Acetylcholine, nitric oxide (NO) and other vasoactive agents dilate pulmonary vessels at birth. During the early hours, the failure of alveolar capillary gas exchange may in part be due to poor perfusion of alveoli, whereas mainly in later stages of RDS, symptoms of pulmonary vascular congestion prevail. Pulmonary hypoperfusion or congestion must be always considered as factors influencing the respiratory course.

In extremely immature lung (<26 wk) the active proliferation of alveolar capillary beds continues [46]. It is not constitutionally expanded and will barely provide a reservoir against hypo-hypervolemia and excessive distending pressures. The growth of both pulmonary vascular beds and the airways are seriously disturbed in lung hypoplasia. Predisposition to PPHN may be the result of acute biotrauma, caused by microbes and inflammatory agents. Increase in pulmonary vascular resistance in generalized acute lung disease is well recognized. This applies to RDS regardless of the gestation at birth.

In extremely premature infants the capacity of pulmonary vessels is low increasing the risk of pulmonary hypoperfusion during continuous distending pressures. These iatrogenic events increase the ventilation-perfusion mismatch, decreasing the gas exchange. Towards term, the muscularity of pulmonary arteries increases. The treatment of RDS in near-term infants is often delayed, allowing progression of respiratory distress and development of PPHN.

62.6.7 Patent Ductus Arteriosus (PDA)

In the fetus, the ductus arteriosus exceeds the diameter of aorta until early in the third trimester. Thereafter the ductus decreases in size in relation to major vessels. Prominent prostaglandin inhibitor-induced contraction of the fetal ductus arteriosus is evident beginning in the third trimester and increasing towards term. After birth, the cascade of events leading to permanent constriction followed by anatomical closure of the ductus takes place in virtually all term born infants, whereas the degree of prematurity progressively increases the risk of PDA.

PDA in VLGA and particularly ELGA continues to be a treatment challenge and an important factor influencing pathogenesis of prolonged respiratory distress. A left-to-right shunt through PDA causes pulmonary congestion at the expense of poor perfusion of other organs. This may happen rather soon after birth in RDS, as the surfactant-induced remission of RDS decreases pulmonary vascular resistance. Several co-morbidities may associate with hemodynamically significant PDA: severe RDS, intracranial hemorrhage, pulmonary edema/hemorrhage, necrotizing enterocolitis, retinopathy of prematurity and particularly BPD. Inhibitors

of prostaglandin synthesis and surgery are traditional therapies, and antenatal glucocorticoid, early neonatal caffeine or hydrocortisone decrease the risk of PDA. Value of several management practices in preventing/decreasing severity of BPD and adverse respiratory consequences (maintaining adequate blood volume while avoiding overhydration, continuous distending airway pressures) have been proposed.

62.7 Prevention of RDS

Elimination of prematurity would be most effective in prevention. There are remarkable variations in the risk of prematurity on the basis of ethnicity, socioeconomic status and the health care system (see Chapter 4). Supplementation of progesterone acetate from mid term pregnancies with previous spontaneous preterm birth has reduced the prematurity rate. However, the long-term risks have not been studied, and administration of progesterone in other indications has been barely successful. *In vitro* fertilization practices increase the risk of premature birth. This is mostly due to iatrogenic multiple births. Obstetric management and even early neonatal management of preterm infants influence the risk and severity of RDS. The practice of antenatal glucocorticoid and rate of elective near-term and term deliveries significantly influence the risk.

Currently, effective therapies of RDS are available and as a result the prognosis is remarkably improved. However, the mean cost of treatment is high and significant mortality continues to be evident even among near-term infants [50]. In very preterm infants RDS is a common disease that influences the risk of both acute (pneumothorax, lung hemorrhage, PDA, IVH, bowel perforation) and chronic morbidity (BPD, neurocognitive problems).

62.7.1 Pharmacological Acceleration of Fetal Maturity

In 1972 Liggins and Howie demonstrated that antenatal glucocorticoid (AGC) given in threatened preterm birth decreased the incidence of RDS and neonatal mortality [51]. This study based on similar evidence in pregnant ewes, was repeated a number of times, but first became fully accepted in the mid-nineties [8]. The concerns of adverse long-term effects have not materialized.

AGC influences the development of the fetal lung, gastrointestinal tract, cardiovascular system, liver, kidney and central nervous system. It accelerates the differentiation of the surfactant system, promotes the absorptive state of fetal lung, enhances the closure of PDA after birth, increases the generally low blood pressure of preterm infants shortly after birth, improves the performance of the immature left ventricle and has multiple potentially beneficial effects on the immune system. Other drugs and combinations of glucocorticoid and thyroid hormone have been studied in clinical trials. However, glucocorticoid is the drug of choice for this indication.

AGC given before 34 weeks of pregnancy in threatened preterm birth, decreases the incidence of RDS when started 1–7 days before the birth. The effect is observed in several high-risk groups. Antenatal glucocorticoid decreases the risks of RDS in preterm infants exposed to chorioamnionitis. This is not unexpected as glucocorticoid and cytokines or endotoxin have an additive effect in stimulating the maturation of the surfactant system.

Most of the studies were performed before introduction of exogenous surfactant. However, AGC and exogenous surfactant supplement each other. ACG increases the efficacy of surfactant in decreasing the respiratory failure and decreases the incidence of IVH in the era of surfactant therapy. The preferred drug is betamethasone (BM: 12 mg i.m. × 2, 24 h after 1st dose); an alternative drug is dexamethasone (6 mg × 4 i.m. q 12 h). According to meta-analysis, glucocorticoid decreased neonatal mortality (RR 0.69, 95% CI 0.58–0.81), IVH (RR 0.54, 95% RI 0.43–0.69), NEC (RR 0.46, 95% CI 0.29–0.74), and tended to decrease neonatal infections (RR 0.83 95% CI 0.66–1.04). No adverse effect on fetal, neonatal or postnatal growth has been observed. The cognitive and neurological problems observed in rodents following AGC have been of concern. However, according to follow-up trials, there is a trend towards improved outcome, if any. Exposure to AGC is associated with insulin resistance in young adults [52]. The follow-up continues.

Repeating the AGC when the premature fetus remains undelivered for longer than one week, has been evaluated. Weekly and bi-weekly repeat treatments or just one repeat dose of betamethasone in threatened preterm birth decreased the risk of RDS. Other beneficial outcomes observed after the single dose were not evident [53]. Repeating the drug weekly decreased the birth weight, including the head circumference. Altogether 20–25% of the fetuses exposed to multiple repeat doses were delivered at term. A single rescue dose of betamethasone before imminent preterm birth caused no growth retardation at birth. Although in follow-up studies no adverse influence on neurologic or cognitive function at the age of two years was evident. Longer follow-up studies are necessary [54] for defining AGC.

62.7.2 Amniotic Fluid Analysis for Fetal Lung Maturity

Lung surfactant secreted into liquid-filled air spaces is carried by the lung liquid, which is partly swallowed and partly accumulated into the amniotic fluid. This amniotic surfactant pool starts emerging with imminent functional maturity of fetal lung. Surfactant increases in concentration and quality

in a characteristic fashion as the pregnancy proceeds. Surfactant indices, measured in the amniotic fluid (lecithin/sphingomyelin ratio, phosphatidylglycerol, so-called lamellar bodies, lung profile) have been used to evaluate the risk of RDS of an unborn fetus, particularly in the era when antenatal glucocorticoid or exogenous surfactant were not used [30]. Assessment of fetal lung maturity in preterm and near-term pregnancies is still indicated when there is a need to balance the risk of continuing a risk pregnancy against the risk of iatrogenic RDS after elective near-term birth. The reported specificity and sensitivity of the surfactant indices in amniotic fluid have ranged from 92–100% and 30–70%, respectively. The accuracy suffers because surfactant in amniotic fluid is not only dependent on lung maturity but also on complex regulation of the volumes of fetal lung liquid and the amniotic fluid that serve as surfactant carriers.

62.7.3 Prevention of RDS in Near-Term and Term Pregnancy

Avoidance of birth asphyxia is a cornerstone of the fetal management. Asphyxia in term or near-term infants is currently a rare cause of RDS or ARDS. However, a significant fraction of near-term infants would develop RDS if delivered before the onset of active labor. This estimate is based on the analysis of surfactant profile from the amniotic fluid specimen and actually confirmed by clinical observations [30]. RDS among patients born at 39 weeks of pregnancy or later is very rare regardless of the mode of delivery whereas the risk of meconium aspiration syndrome continues to increase.

In elective near-term births without onset of labor, the fetus is exposed to increased risk of transient tachypnea, spontaneous pneumothorax, RDS, and persistence of fetal circulation. The induction of the adsorptive state of lung liquid secretion prevents the disappearance of secreted alveolar surfactant from the lung and results in accumulation of surfactant in lung liquid, particularly in alveoli. The levels of serum cortisol and adrenalin that induce the adsorptive state increase towards term, particularly during labor. Since the hormones additionally increase the secretion of surfactant, the labor decreases two major risk factors. According to available trials antenatal glucocorticoid in near-term pregnancies decreases the risk of respiratory distress. However, before this treatment is accepted as a therapy, further trials evaluating the efficacy and safety are required. It is imperative to avoid iatrogenic prematurity when it is safe to fetus and mother.

In pregnancies complicated by maternal diabetes, severe metabolic disease, acute bleeding (total placenta previa) or bleeding disorders, placental diseases causing IUGR or hemodynamic compromise of the fetus, in serious isoimmunization and some other rare diseases, elective near-term delivery is indicated, as the risk of continuing the pregnancy balance against early delivery.

Since RDS and the other forms of respiratory distress are relatively rare diseases in near-term infants, expanding routine indications of AGC beyond the 34th week of pregnancy remains to be critically evaluated. Balancing the benefits and potential risks (behavioral problems, increased risk of metabolic syndrome in later life among the exposed infants) contradicts against the use of AGC on a large scale, unless the long-term data on safety is available. In some near-term births ACG is indicated when the risk of respiratory distress is considerable. These cases include maternal diabetes, severe Rh immunization, diaphragmatic hernia, previous near-term RDS in the family and documentation of fetal lung immaturity.

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63

Pulmonary Hemorrhage, Transient Tachypnea and Neonatal Pneumonia

Richard J. Martin and Amitai Kohn

63.1 Pulmonary Hemorrhage

In newborn infants, pulmonary hemorrhage, often a manifestation of pulmonary edema can range in severity from blood-tinged secretions in the endotracheal tube to life threatening blood loss with hypovolemic shock. It usually presents in the second to fourth day of life and may be associated with lung tissue damage (RDS, infection, and mechanical ventilation with high-inspired oxygen), hypoxia, hypervolemia, hypoproteinemia, congestive heart failure, and coagulation abnormalities. Klukow confirmed an association between pulmonary hemorrhage and a large patent ductus arteriosus with high pulmonary blood flow [1].

Pulmonary hemorrhage occurs when a build up of filtrate containing plasma and whole blood produces hemorrhagic edema fluid and results in an increased capillary pressure [2] When the pressure cannot be contained, the resultant injury leads to an acute hemorrhagic picture. Erosion or ulceration following an injury to the upper airway is another mechanism by which bleeding into the lung can occur.

Pulmonary hemorrhage was present in the lungs of up to 68% of infants who died in the first week of life and associated with the need for cardiopulmonary resuscitation in the neonatal intensive care unit [3]. In 9% of neonatal autopsies, pulmonary hemorrhage was the principal cause of death and a major risk of mortality associated with meconium aspiration syndrome. Of the total population of VLBW infants, 5.7% had pulmonary hemorrhage with a high associated mortality, possibly related to gasping during labor [4].

The clinical picture of pulmonary hemorrhage is quite distinct. The infant may present with signs of hypovolemic shock, cyanosis, and apnea. Red or pink tinged secretions are suctioned from the orophrynx or endotracheal tube and, at any time, secretions may turn into massive bleeding. The radiographic picture constitutes patchy infiltrates as seen in Fig. 63.1.

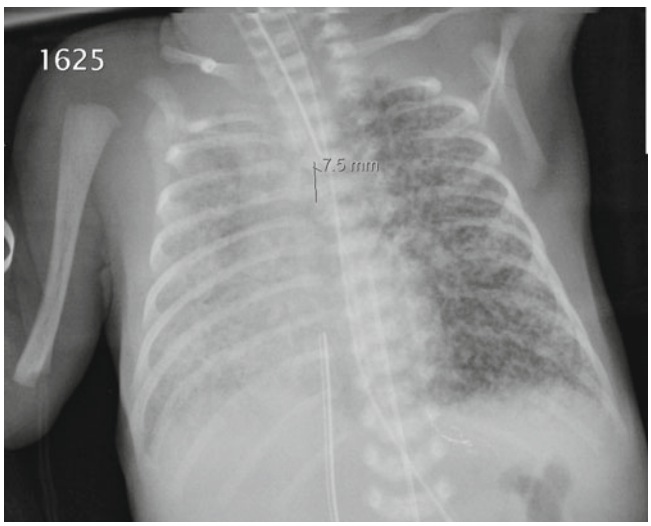

Fig. 63.1 Diffuse, patchy infiltrates and right-sided atlectasis caused by pulmonary hemorrhage

The management of pulmonary hemorrhage comprises maintaining cardiac output, transfusion of blood products as necessary and correction of acidosis. The initiation of mechanical ventilation with increase in PEEP or PIP may potentially tamponade small pulmonary vessels. In some instances, high frequency ventilation may also be helpful, possibly by maintaining a high mean airway pressure. Furthermore, several studies have suggested that closure of the patent ductus arteriosus with early medical intervention may reduce the risk of pulmonary hemorrhage. It is widely believed that pulmonary hemorrhage in the preterm infant is secondary to a patent ductus arteriosus and resultant pulmonary edema in the majority of cases.

While surfactant therapy has been implicated as one of the possible etiologies for pulmonary hemorrhage, it also has

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

a therapeutic role in the management of the same phenomenon [5–10]. This is due to the possible inactivation of surfactant production by the hemorrhagic fluid.

In a recent small study, the introduction of hemocoagulase via the endotracheal tube every 4–6 hours in addition to mechanical ventilation increased survival, decreased the length of pulmonary hemorrhage and decreased the need for prolonged mechanical ventilation due to the pulmonary hemorrhage [11]. The outcome of pulmonary hemorrhage depends on the underlying etiology and the severity of the infant's underlying cardiorespiratory status.

63.2 Transient Tachypnea of the Newborn

Transient tachypnea of the newborn (TTN) was originally described by Avery et al in 1966 as the clinical manifestation of delayed clearance of fetal lung fluid [12]. In her work, Avery characterized early onset of respiratory distress in eight late premature infants with radiographic findings of lung hyperinflation, increased pulmonary vascular markings and cardiomegaly. Symptoms were mild and transient with infants improving in a 2–5 day period. Until recently, it was believed that once TTN resolves, there is no added respiratory morbidity or long-term effect on the infant [12–14]. However, a recent large retrospective study of term infants suggested that TTN might be significantly associated with childhood asthma with increased propensity in males. This study also suggested TTN as a marker of diminished pulmonary function, which may reflect an inherited susceptibility to asthma [15].

The incidence of TTN is approximately 1% of live births. The risk for developing TTN seems to increase with prolonged labor with failure to progress, cesarean section, prematurity, birth of a male infant, and macrosomia [16, 17]. Prolonged maternal administration of hypotonic fluid such as during prolonged labor, decreased resorption of lung fluid in the neonate, and maternal asthma have all been suggested as a cause for TTN [13, 18, 19].

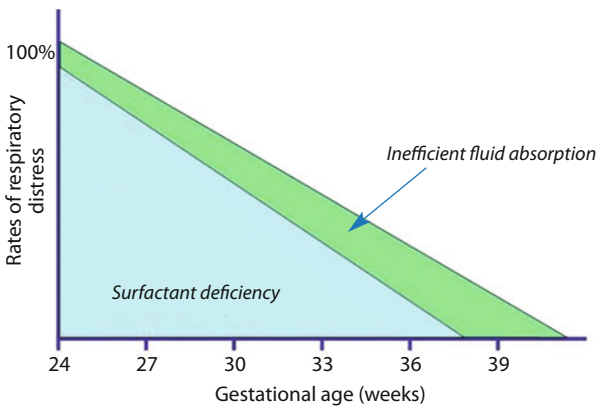

Fig. 63.2 Relative contribution of surfactant deficiency and insufficient fluid absorption [TTN] to neonatal respiratory distress. Adapted from [28]

Most of the published work related to the pathophysiology of TTN has sided with Avery's conclusions that TTN is the result of delayed fetal lung fluid clearance [20, 21]. The lung's functional residual capacity comprises a potential air space of 20–30 mL/kg body weight. This space is filled in utero with fetal lung fluid containing high potassium and chloride, and low bicarbonate and protein. Shifts between the body compartments are controlled by a chloride active pump [15, 22–25]. Two to three days prior to delivery, the fetus begins its lung fluid clearance in anticipation of transition to extrauterine life. This process begins with a decreased rate of fetal lung fluid secretion. The major clearance takes place with the onset of labor. At this time, the lung epithelium becomes a sodium absorbing membrane and lung fluid flow is directed from the air space to the intersititum. In addition, low protein containing lung fluid exhibits low oncotic pressure, which is another driving force for lung fluid to enter the vascular system.

Mechanical compression via vaginal delivery may also play a role in lung fluid clearance. Milner et al [26] showed that when infants were not delivered through the vaginal canal and were not exposed to vaginal compression they had higher interstitial and alveolar fluid volume. Furthermore, these infants also had a decrease in their lung gas volume. This hypothesis is not supported by later animal and human studies that showed no increase of functional residual capacity (FRC) in vaginally delivered infants [20, 27]. Results by Birnkrant and a retrospective study by Liem [15, 21], that found an association between TTN and the development of wheezing syndromes, support the pathophysiologic hypothesis of fetal lung fluid clearance at the cell pump level and not the mechanical compression theory.

The common clinical picture is of a term or a late premature infant with possible mild birth depression, who shortly after birth presents with respiratory distress including tachypnea, grunting, nasal flaring, subcostal retractions, and possibly cyanosis. The maternal history is often positive for maternal anesthesia, maternal diabetes, or cesarean section. Arterial blood gas is likely to show mild respiratory acidosis with potential hypoxemia. Chest radiographs typically demonstrates perihilar streaking which may be due to periarterial lymphatic engorgement. TTN may also present in premature infants. In this group of infants, TTN and pulmonary edema may further complicate surfactant deficiency and increase the likelihood of requiring exogenous surfactant and ventilator support. With advancing gestation and in the late preterm infant, TTN increasingly contributes to the pathophysiology of respiratory distress when compared to surfactant deficiency [28].

TTN is transient in nature and the resolution of symptoms is usually within 8–12 hours but may take up to 5 days. Infants may require oxygen support but, rarely over 40% inspired oxygen. In the case of respiratory acidosis, CPAP will support ventilation. Due to difficulty in distinguishing TTN from pneumonia, infants may receive a course of antibiotics. The radiographic findings of TTN should spontaneously resolve by 48 hours [13]. In the past, furosemide was thought

to help in the fluid clearance, however in a randomized control trial of oral furosemide there was no significant difference between treated and control TTN groups.

63.3 Neonatal Pneumonia

Lungs are a major site of origin and location for sepsis in the newborn. Pneumonia may be acquired pre- or postnatally and be of bacterial, viral, fungal or protozoan origin. Morbidity is high which should lead to high alertness when facing an infant with signs of respiratory distress. An immature immune system and poor mechanical defense mechanisms may increase susceptibility to invasion of pathogens into the lungs. Furthermore, when an infant has added morbidity such as respiratory distress syndrome (RDS), meconium aspiration syndrome or chronic lung disease (CLD), both vulnerability to and consequences of pneumonia may be enhanced.

The incidence of neonatal pneumonia is clearly much higher for preterm infants then term infants. Barnett and Klein [29] reported intrauterine and early onset pneumonia in 10–38% of stillbirths and in 20–63% of autopsies from live births that died in the first 28 days of life. The difficulty of reporting incidence is in the inconsistency of defining pneumonia in infants less than 1 month of age. The majority of late onset pneumonias are diagnosed in premature infants and most were on ventilatory support at the time of diagnosis. A small single center study by Apisarnthanarak [30] showed 28% of ventilated infants developed ventilator associated pneumonia (VAP) and in 1986, Halliday [31] reported a 35% incidence of pneumonia in intubated patients with RDS. Despite efforts by the Center for Disease Control (CDC) and National Nosocomial Infection Surveillance System (NNIS) there is still not a gold standard for diagnosing VAP [32, 33]. This adds to the major discrepancies between studies that report rates between 10–35% [34–36].

The etiology of neonatal pneumonia can be divided into 3 categories: congenital, early onset and late onset. Congenital or intrauterine pneumonia is usually caused by ascending infecting organisms from the maternal urogenital tract before or during pregnancy, or via the transplacental route. Prolonged rupture of membrane increases the risk of bacterial acquisition by the newborn however; this is also seen with intact membranes. Microorganisms such as viruses (cytomegalovirus, rubella, herpes simplex virus, adenovirus, varicella zoster virus, entroviruses and influenza A), bacteria (*Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Treponema pallidum*), and protozoa (*Toxoplasma gondii*) are known causes of intrauterine pneumonia. However, these microorganisms cause multi-organ inflammation of which pneumonia represents a variable component.

Early onset pneumonia is usually due to introduction of bacteria from the mother's vaginal tract during the delivery process especially in a situation of prolonged membrane rupture [37, 38]. Gasping due to asphyxia and/or meconium aspiration may introduce organisms into the respiratory system or be a consequence of infection in the fetus. Other factors such as prematurity and maternal urinary tract infection have a major role in increasing the risk of neonatal pneumonia. Group B streptococcus [GBS] is one the major causes of pneumonia in the neonate [34]. Overt GBS sepsis is seen in 1% of colonized infants or 1–4/1000 live births if no intrapartum prophylaxis with antibiotics is given. Premature infants comprise one third of all infants with GBS bacteremia [39]. In 1993, committees of the American Academy of Pediatrics published guidelines for intrapartum chemoprophylaxis of group B streptococcal positive mothers [40]. These guidelines were revised in 1997 and have proven successful to date with a significant decrease in GBS sepsis to 0.6 per 1000 births in 1998 [41]. In 2002 [42], the Centers for Disease Control and Prevention (CDC) in the USA revised the prevention of perinatal Group B streptococcal disease guidelines concentrating on prenatal screening, updated prophylaxis regimens, prenatal specimen collection, culture methods and susceptibility testing. In addition, the CDC argued against routine intrapartum antibiotic prophylaxis for GBS colonized women delivering by planned cesarean section prior to the onset of labor or rupture of membranes. They suggested algorithms for management of patients at risk for preterm delivery as well as infants exposed to prophylaxis treatment. Unfortunately, other microorganisms known to cause early onset pneumonia have increased in importance since the implementation of the GBS guidelines. The common pathogens that may influence the infant in the immediate postpartum period are *Escherichia coli*, *Kelbsiella* spp, *Proteus mirabilis*, *H. influenzae*, Group D streptococci, *Listeria monocytogenes* and pneumococci. In addition, non-bacterial pneumonia may be seen in the early postnatal period such as caused by *Candida* [43, 44], viruses [45–47], and *Chlamydia* [48].

Late onset pneumonia is diagnosed when symptoms arise at or after 48 hours of life. The pathogens are commonly acquired from the environment and are nosocomial. Late onset pneumonia is more common in premature infants or infants on prolonged ventilatory support [30, 34, 49]. Gram-negative bacteria (*E. coli*, *Serratia marcescens*, *Proteus* spp, *Klebsiella* spp, *Pseudomonas* spp), coagulase-negative staphylococci [50, 51] and *Staphylococcus aureus* along with GBS are among the most common bacterial organisms isolated in late onset pneumonia. Viral organisms such as CMV [45, 52] VZV, RSV, parainfluenza, influenza A and B [53], rhinovirus [54], enterovirus [55], and coronavirus are also seen in this type of pneumonia. In addition, fungal etiologies have been implicated. Infections are acquired mainly through skin colonization and breakdown, gastrointestinal translocation of organisms, and from the respiratory tract of family and care providers. The colonization of an infant in the intensive care unit with common or unusual flora can be due to a weak immune system, health care provider exposure, and interventions (endotracheal tube, mechanical ventilation, and multiple courses of antibiotics) [56–59].

Ventilator associated pneumonia (VAP) is a nosocomial bacterial pneumonia that has developed in patients who are receiving mechanical ventilation. VAP can occur at any time and is defined as early or late, based on the timing as mentioned earlier [60]. While similar to neonatal VAP in many aspects, most diagnostic criteria and work up guidelines are based on adult studies. In the neonatal population the clinical and laboratory signs of VAP are nonspecific [61].

It is difficult to diagnose pneumonia with great certainty. Isolation of bacteria or viruses from the trachea or the oropharynx does not necessarily correlate with invasive infection and may just reflect colonization. Radiographic studies may suggest a focal pneumonia, but this presentation is not common and atelectasis may be difficult to differentiate from infiltrate secondary to pneumonia. The work up for suspected neonatal pneumonia whether congenital, early or late is similar. Blood, and airway cultures (nasophayngeal or tracheal if intubated) should be obtained and may be positive for the same microorganism in the case of respiratory infection [58]. Sherman [62], showed that tracheal secretions with a positive Gram stain in relation to neonatal bacteremia had a 74% sensitivity and 47% positive predictive value and concluded that gram stain of tracheal secretions may be of practical value in the diagnosis of congenital bacteremia. Positive airway culture alone may be more suggestive of early than late onset pneumonia, however in neither case is it sensitive [63, 64]. In the case of late, ventilator associated pneumonia (VAP), Mayhall [65] reviewed the diagnostic procedures for VAP based on the CDC (1997) Guidelines [66], although, these guidelines were intended for adult patients and are not specific for the neonatal population. Isolated positive tracheal culture did not distinguish between bacterial colonization and respiratory infection [61]. Radiographic studies are frequently non-specific, and it is more common to find pleural effusions in bacterial and fungal infections. Other non-specific laboratory values such as C- reactive protein (CRP) and WBC counts, especially immature to mature neutrophils ratio (I/T) may aid in the diagnosis of pneumonia.

The difficulty in diagnosing pneumonia especially when it coincides with RDS, presents the clinician with a therapeutic dilemma. Low absolute neutrophil count (ANC), high I/T ratio (>0.2) and CRP (>1) have shown to have positive predictive value of bacterial sepsis [66, 67]. Leslie et al [68] showed high I/T, low total neutrophil counts and positive gram stain to be more consistent with early onset bacterial pneumonia than RDS. However, diagnostic difficulty remains and if the index of suspicion for sepsis is high, the accepted treatment route is antibiotics for 48 hours while awaiting culture results and following the above markers trends. It is also accepted to treat every infant who deteriorates from a respiratory standpoint especially if the infant was asymptomatic for the first 6 hours of life.

The recommended empiric choice for early onset pneumonia is ampicillin in conjunction with an aminoglycoside (gentamicin). For late onset pneumonia, empiric therapy may constitute nafcillin and an aminoglycoside. Vancomycin is used empirically in extremely sick infants or infants deteriorating in spite of antibiotic treatment. When cultures are definitive and sensitivities are known appropriate antibiotics should be used. The prognosis of neonatal pneumonia depends on the underlying etiology and overall condition of the infant.

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64

Pulmonary Air Leakage

Paola Papoff and Corrado Moretti

64.1 Pulmonary Air Leaks

Pulmonary air leaks include a number of clinical conditions characterized by overdistension and rupture of the alveolar wall with air leaking from the intra-alveolar space into the surrounding tissue. Air leaks are more common and more severe among neonates with pulmonary diseases that are characterized by poor lung compliance (e.g., respiratory distress syndrome) or by airway obstruction (e.g., meconium aspiration syndrome).

In neonates, the most common air leaks are pulmonary interstitial emphysema, pneumomediastinum and pneumothorax, while pneumopericardium, pneumoperitoneum and venous pulmonary embolism are less frequent. Affected neonates may be asymptomatic or have various degrees of respiratory distress. Diagnosis is suspected clinically because of respiratory distress and increased oxygen requirement and confirmed by X-ray. Treatment varies by type and severity of air leak.

64.2 Structure of the Lung

Macroscopically the lung is divided into three lobes on the right and two on the left, each of which have autonomous ventilation and blood supply. Each lobe comprises segments, which are pyramidal in shape with the apex towards the hilum and the base towards the surface of the lung. The segments are supplied by a bronchus and by a segmentary artery and are drained by a perisegmentary venous network. Each segment comprises pulmonary lobules, each of which is supplied by a lobular bronchiole, and a branch of the pulmonary artery.

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The lobular bronchiole continues branching until it reaches the level of terminal bronchioles, which ventilate the alveolar sacs that comprise clusters of alveoli. There are 10–15 alveoli in each alveolar sac. These are the functional units of the lung where gas exchange occurs.

The bronchial tree and associated vessels are wrapped in a sheath of collagen and elastic tissue. The pressure on the pleural surface is transmitted through the alveolar walls to the alveoli. Each alveolar unit is interdependent with the adjacent one so that the expansion of one alveolus determines the increase in wall tension of neighboring alveoli and ensures uniform pulmonary expansion.

Most of the development of the pulmonary elastic structure occurs during the fetal saccular period, which begins towards the 25th week of gestation.

The terminal bronchiole gives rise to two or more respiratory or alveolar bronchioles, which develop hemispheric evaginations called alveoli. Alveoli are more numerous in the distal portion of the respiratory bronchiolus, which ends by branching into alveolar ducts and finally the alveolar sacs, which form the terminal portion of the respiratory airways.

Each lung is enveloped by a serous membrane, the pleura.

64.3 Pulmonary Interstitial Emphysema

Pulmonary interstitial emphysema (PIE) may be defined as a collection of gas outside the airway and inside the interstitial space. It is caused by alveolar and bronchiolar rupture. The extent of PIE can vary. PIE may occur throughout the lungs or may be unilateral or lobar. In the former case, it may be difficult to differentiate PIE from early bronchopulmonary dysplasia (BPD).

The incidence of PIE has decreased because of treatments such as surfactant and the concept of "gentle ventilation", as well as the availability of high-frequency oscillatory ventilation (HFOV) for infants who are difficult to maintain with conventional ventilation [1].

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Risk factors for PIE are:

- Mechanical ventilation with high pressures (the most important factor)
- Low gestational age (PIE usually develops in infants younger than 32 weeks' gestation, who weigh less than 1200 g) [2]
- Pulmonary hypoplasia
- Pulmonary diseases, e.g., severe respiratory distress syndrome (RDS), meconium aspiration syndrome (MAS), infectious or aspiration pneumonia [3]
- Chorioamnionitis and funisitis [4]
- Malpositioned endotracheal tube [5].

64.3.1 Etiology and Pathogenesis

The mechanisms involved in spontaneous, interstitial, mediastinal and subcutaneous emphysema have been described by Macklin and Macklin [6]. Their experimental work with various animal models led to the hypothesis that overdistended alveoli rupture into the pulmonary vascular sheaths and create PIE. Predisposing factors for PIE are positive pressure ventilation (PPV) with high mean airway pressures (MAP) and reduced lung compliance.

The basic requirement for rupture is the existence of a pressure gradient between the alveolus and its surrounding structure. However, in extremely premature infants, PIE can occur at low mean airway pressures and probably reflects increased sensitivity to stretch by the immature lung. The pressure within adjacent alveoli is generally assumed to be similar and the interalveolar wall remains intact. As illustrated schematically in Fig. 64.1, if a pressure difference develops between the alveoli, the risk of alveolar rupture is increased, causing interstitial emphysema. Since the mean pressure within the mediastinum is always rather lower than in peripheral lung parenchyma, air dissects proximally along the bronchovascular sheaths to the lung hilum and mediastinal soft tissue. Having reached the mediastinum, accumulated air may decompress into cervical and subcutaneous tissue or to the retroperitoneum, dissecting along the fascial planes. If mediastinal pressure rises abruptly or decompression via these routes is not sufficient to relieve the tension, the mediastinal parietal pleura may rupture, resulting in pneumothorax. This mechanism, rather than rupture of subpleural blebs, is thought to be responsible for spontaneous pneumothorax in a majority of cases [8].

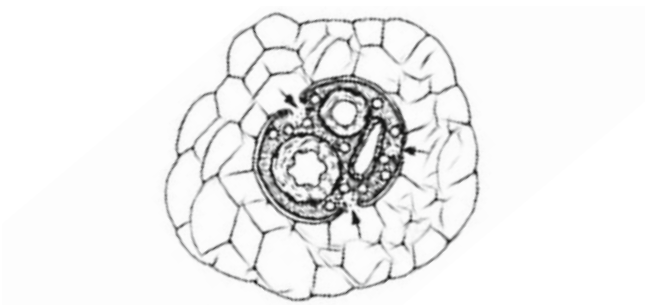

Fig. 64.1 Distal bronchovascular sheath surrounded by alveoli after expiratory airflow obstruction. Pressure gradient between airspace and perivascular interstitium leads to alveolar rupture (*arrows*) and direct introduction of air into bronchovascular sheath. Modified from [7]

The presence of air in the interstitial tissue causes reduced compliance, an increase in residual volume and dead space, and an increased ventilation perfusion (V/Q) mismatch. It also impedes pulmonary blood flow and obstructs the lymphatic drainage. These changes account for worsening oxygenation.

At an early stage of RDS, there is increased interstitial and perivascular fluid that rapidly decreases over the next few days. This fluid may obstruct the movement of gas from ruptured alveoli to the mediastinum through the bronchovascular sheaths, causing an increase of PIE. Another possible mechanism for entrapment of air in the interstitium is the increased amount of connective tissue in the immature lung. Entrapment of air in the interstitium may result in a vicious cycle that causes compression of the adjacent alveoli and a further increase in ventilation pressure with more air escaping from the overdistended alveoli into the interstitial tissues.

64.3.2 Clinical Aspects, Differential Diagnosis and Prognosis

PIE may occur during the first 72 hours of life or as a complication of prolonged positive pressure ventilation in older premature infants.

The clinical presentation of PIE varies with size and location of gas accumulation. PIE may cause compression of small airways with wheezing or diminished breath sounds because of decreased lung compliance. There may be changes in ventilator requirements, increased respiratory rate, and variations in heart rate and blood pressure. Trapped gas reduces pulmonary perfusion by compressing blood vessels, causing hypoxemia, hypercapnia and respiratory acidosis.

The air collection along the bronchovasal sheaths is visible radiologically as small rounded, nonconfluent, microradiolucencies throughout the lungs (Fig. 64.2). PIE may be unilateral (Fig. 64.3). If there is mechanical ventilation and because of rupture of interlobular septa, PIE tends to form hypertransparent bubbles that may be quite large and look like pseudocysts. Small areas of PIE alternating with pneumatoceles have the effect of making the pulmonary parenchyma look like a honeycomb.

Possible complications of PIE include other air leaks, such as pneumomediastinum, pneumothorax, pneumopericardium, pneumoperitoneum, subcutaneous emphysema, and pulmonary venous embolism, intraventricular hemorrhage, and possible epithelialization of the interstitial air pockets.

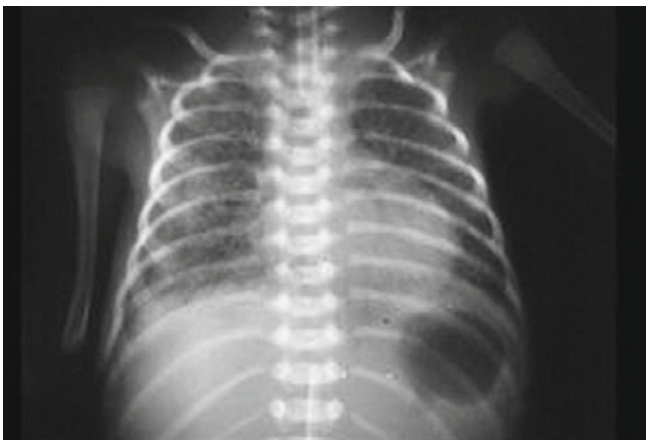

Fig. 64.2 Diffuse pulmonary interstitial emphysema

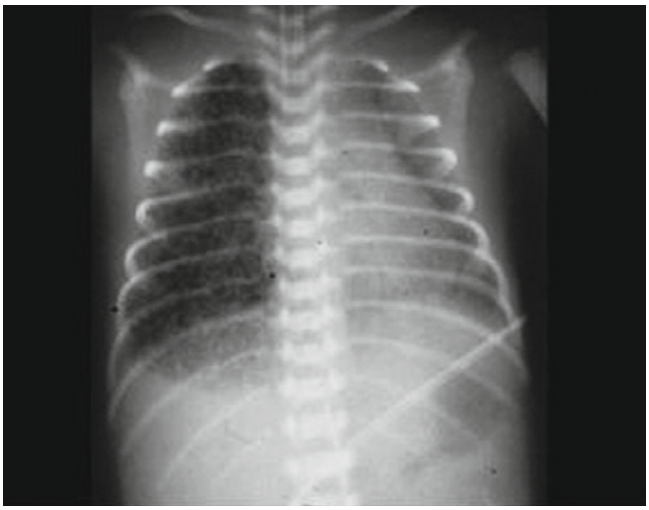

Fig. 64.3 Pulmonary interstitial emphysema, especially marked in the right lung

PIE may be confused with severe RDS, early BPD, cystic adenomatoid malformation, lymphangiectasia, bronchogenic cysts, congenital lobar emphysema, and pulmonary infection. PIE may take several weeks to resolve. The condition can therefore increase the length of time of mechanical ventilation and the incidence of BPD [2]. Some infants may develop chronic lobar emphysema and may require surgical lobectomies.

64.3.3 Therapy and Treatments

In mechanically ventilated infants, initial treatment of PIE should be aimed at obtaining maximum patient-ventilator synchrony, securing the endotracheal tube in the appropriate position, and reducing peak inspiratory pressure (PIP) and positive end expiratory pressure (PEEP) as much as possible, consistent with blood gas values. A short inspiratory time (Ti) and a triangular pressure wave morphology should be set to avoid inflating the parenchymal cysts that have a long time constant [9]. HFOV has been found to be effective in the treatment of PIE [1].

Treatment of the localized form includes positioning the infant with the affected side down or selectively intubating the main bronchus of the unaffected side [10, 11]. Severely ill premature neonates may not tolerate the latter treatment because it is difficult for them to compensate for distress with one functioning lung. Physiotherapy may be useful as the presence of mucus may contribute to the formation of cysts and bubbles through a valve mechanism [12]. A short course of dexamethasone has been shown to be as an effective treatment in infants with PIE, possibly by reducing airway edema and inflammation and airway obstruction [13].

64.4 Pneumomediastinum

Pneumomediastinum is defined as a collection of gas in the mediastinal space. Free air may not be confined solely to the mediastinum but may penetrate through tissue planes, causing pneumopericardium, pneumothorax, subcutaneous emphysema, or pneumoperitoneum. Pneumomediastinum occurs in approximately 2.5 per 1000 live births.

Risk factors for pneumomediastinum are:

- Gas trapping associated either with several lung diseases, including RDS, pneumonia, MAS or with mechanical ventilation
- Birth asphyxia requiring resuscitation
- Chest trauma
- Mechanical obstruction as seen with a foreign body or a tumor.

64.4.1 Etiology and Pathogenesis

Pneumomediastinum is in most cases a complication of a pre-existing pulmonary disease and may be preceded by PIE. The air that leaks out of the alveoli makes its way along the bronchovasal sheaths toward the hilum and into the mediastinum. Pneumomediastinum may appear spontaneously at birth or may occur later, secondary to the formation of a valve-like mechanism and consequent rupture of the alveoli. The air moving from the mediastinum towards the neck causes the dissection of the cervical aponeurosis and the supraclavicular tissue planes, forming subcutaneous emphysema. The air in the mediastinum may also spread to the sub-pleural space and simulate a basal pneumothorax. Air may also leak into the peritoneal cavity through areas of lower resistance, such as the sternocostal trigone, and cause subdiaphragmatic air collections (pneumoperitoneum).

64.4.2 Clinical Aspects and Differential Diagnosis

Pneumomediastinum rarely leads to clinically significant complications. However, compression of mediastinal structures may occur when there is extensive subcutaneous and mediastinal gas. The infant with pneumomediastinum is usually asymptomatic or has mild respiratory distress. In the absence of other pulmonary disorders it tends to regress spontaneously.

A large accumulation of trapped air may cause severe respiratory distress and reduced cardiac output. The first sign may be subcutaneous emphysema in the neck, face, or chest. The infant with a large pneumomediastinum may exhibit bulging in the midthoracic area, distended neck veins, and low blood pressure.

The pneumomediastinum is characterized radiologically by the presence of cardiac margins that are clearly delimited by a thin black streak. The edges of the right atrium and the left ventricle are generally well distinguished from the pulmonary parenchyma. In cases of pneumomediastinum with small quantities of air, the only sign is a radiolucent band (hyperlucency) in the retrosternal area on the lateral view (Fig. 64.4) [6, 14]. The main diagnostic element of pneumomediastinum is prominence of lobes of the thymus that may be more or less marked depending on the amount of air in the mediastinum (the image resembles the wings of a sea-gull or a spinnaker sail, Fig. 64.5) [15, 16]. When there is a pneumomediastinum, the lobes of the thymus are surrounded by air and in the lateral view this peculiar radiographic aspect is sufficient to diagnose an antero-medial pneumothorax. The air in the mediastinum may push both thymus lobes toward the pulmonary apices, thus simulating upper lobar collapse or pneumonia. The presence of other signs suggesting pneumomediastinum allows this condition to be differentiated from pulmonary consolidation.

Air may collect in some mediastinum recesses, such like the triangular ligament. In such cases, the chest X-ray shows a hyperlucent paravertebral area extending from the diaphragm up to the pulmonary hilum (Fig. 64.6) [17].

Pneumomediastinum should be differentiated from bronchogenic cysts [18], esophageal perforation and pneumothorax.

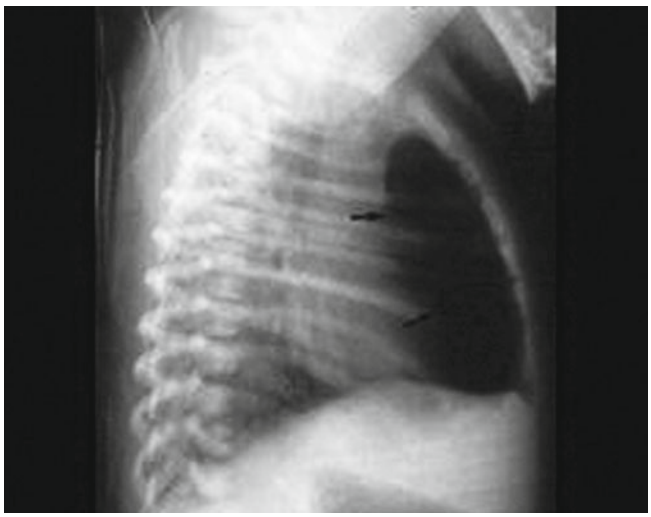

Fig. 64.4 Pneumomediastinum, lateral view

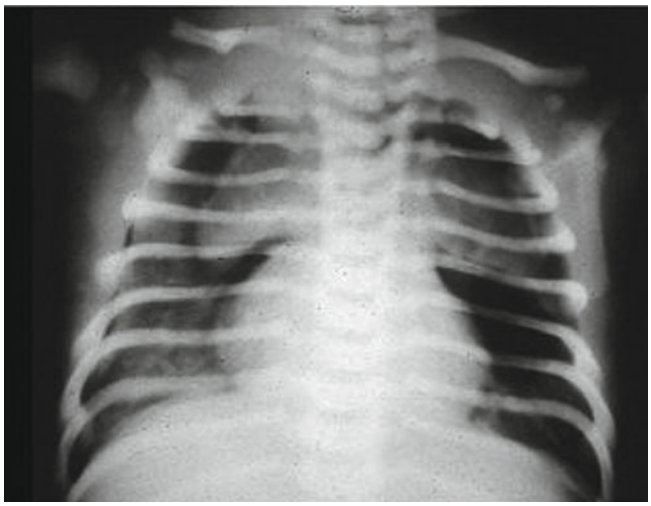

Fig. 64.5 Pneumomediastinum with the raised thymus lobes (spinnaker sail)

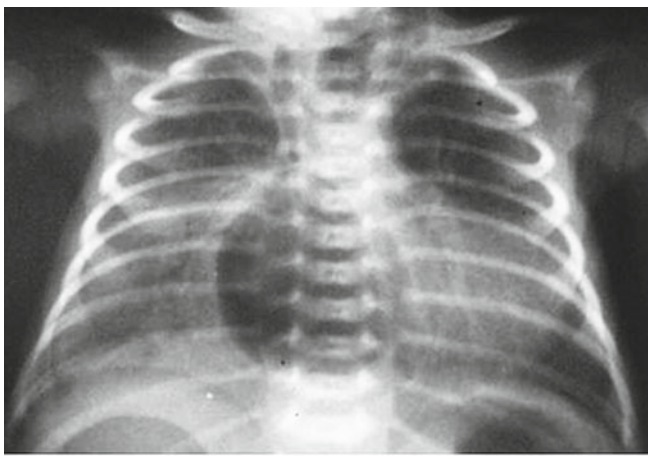

Fig. 64.6 Pneumomediastinum with the air collected in the triangular ligament

64.4.3 Therapy and Treatments

A conservative approach is usually adopted. Treatment with 100% oxygen may accelerate air reabsorption. High inspired O_2 concentrations facilitate nitrogen washout from the bloodstream (PN_2 drops from 573 mmHg at room air to 0 mmHg under 100% oxygen) creating a concentration gradient between pleural gas and pleural venous circulation. This gradient favors the reabsorption of nitrogen within the pleural pockets. This technique should not be applied to very low gestation infants because of the risk of oxygen toxicity.

64.5 Pneumothorax

Pneumothorax is defined as an accumulation of air in the pleural space. Pneumothoraces have been termed small, moderate or large if they occupy 15% or less, 15%–60%, or more than 60% of the pleural space.

Spontaneous pneumothoraces develop in up to 1% of normal term infants around the time of birth (only about 10% of these are symptomatic) [19]. A likely cause is because of high transpulmonary pressure during the first breaths. In the presence of lung disease, especially if mechanical ventilation is required, the incidence of pneumothorax increases dramatically (~10%). Fifteen–20% of pneumothoraces are bilateral. Two thirds of unilateral pneumothoraces affect the right side. There is no correlation between the incidence of pneumothorax and gestational age [5]. Reports of familial spontaneous pneumothoraces in neonates are rare [20].

Risk factors for pneumothorax are:

- Lung diseases, such as wet lung (following cesarean section) [21], MAS, congenital bullae, and pulmonary hypoplasia, which result in uneven lung compliance and alveolar overdistension
- Pulmonary interstitial emphysema (PIE)
- Ventilatory support. Predisposing factors during mechanical ventilation are:
  - lack of synchronization between the mechanical cycle of the ventilator and the spontaneous respiratory activity of the infant. Asynchrony is usually secondary to trigger delay, long inspiratory times (Ti) and an inspiratory/expiratory ratio (I/E) ≥ 1:1 [22]
  - high ventilation pressures
  - high PEEP [23]
  - high ventilation tidal volumes (more important than increase in pressure) [24]
  - intubation of the right main bronchus
- Nasal CPAP (the risk is lower than during mechanical ventilation)
- Low temperature of inhaled gases (<36.5°C). In infants weighing ≤1500 g at birth the incidence of pneumothorax increases if the temperature of inhaled gases is below 36.5°C. It is likely that lower temperatures cause a reduced content of water in the ventilation gas, which alters the muco-ciliary clearance [25] precipitating airway obstruction
- Direct injury (suction catheter and central venous catheter positioning).

64.5.1 Etiology and Pathogenesis

In an overdistended lung, air passes from a ruptured alveolus up the bronchovascular sheath into the mediastinum and from there into the pleural cavity causing pneumothorax. The pathogenesis of pneumothorax is comparable to that of the pneumomediastinum. Rarely pneumothorax is the result of the rupture of sub-pleural blebs.

In the neonate, the susceptibility to pulmonary rupture is further enhanced by a reduced number of alveolar connecting channels (pores of Kohn) that allow air to redistribute between ventilated and nonventilated units.

64.5.1.1 Classification

Pneumothoraces are commonly classified into three types: simple, communicating, and tension. Simple or noncommunicating pneumothorax occurs when there is no direct communication with the atmosphere. There is no mediastinal shift resulting from the accumulated air. A simple pneumothorax may occur spontaneously or as a consequence of barotrauma or volutrauma during positive pressure mechanical ventilation.

Communicating or open pneumothorax occurs when there is an association with an open defect in the chest wall, most commonly occurring as a complication of surgery, e.g., PDA surgery. As intrathoracic pressure is less than atmospheric pressure during spontaneous breathing, air rapidly accumulates in the pleural space. Loss of negative intrathoracic pressure results in varying degrees of lung collapse and further respiratory compromise.

Tension pneumothorax is caused by progressive accumulation of air in the pleural space. This collection of air shifts the mediastinum to the contralateral hemithorax and compresses the contralateral lung and great vessels, compromising both cardiovascular and respiratory function. Whether air enters the pleural space through a defect in the chest wall, a lacerated bronchus, or alveolus, a one-way valve effect is created so that air enters during inspiration but cannot escape during expiration. Accumulation of air continues until the intrathoracic pressure of the affected hemithorax equilibrates with atmospheric pressure. At this point, the accumulation of pressure within the thorax leads to collapse of the ipsilateral lung and displacement of the mediastinum (and associated great vessels) towards the contralateral hemithorax. While the superior vena cava is able to move to some extent, the inferior vena cava is relatively fixed within the diaphragm and will be distorted. As two-thirds of the venous return in children comes from below the diaphragm, compression of the inferior vena cava leads to a dramatic reduction in venous return to the heart and cardiovascular collapse.

64.5.2 Clinical Aspects

Diagnosis of pneumothorax is based on clinical signs, physical examination, arterial blood gases, transillumination, and imaging. Pneumothorax presents with signs of respiratory distress; chest asymmetry, episodes of apnea and bradycardia, tachycardia, and hypotension may also occur. During mechanical

ventilation, pneumothorax is frequently tension accompanied by compression of the mediastinal structures, which may cause impaired venous return and cardiocirculatory collapse [26]. On auscultation of the chest, breath sounds are decreased or absent in the affected side and it may be difficult to identify the heart sounds. A pneumothorax should be suspected when there is sudden worsening of the clinical condition. However, its exact location by auscultation is not always as straightforward, especially in very low weight infants. Arterial blood gases may show a respiratory or mixed acidosis and hypoxemia.

Transillumination is an effective method for demonstrating increased transmission of light on the affected side [27]. However, chest X-ray remains the gold standard for the diagnosis of pneumothorax. Large pneumothoraces are generally easy to identify radiologically. The affected hemithorax is larger than the contra-lateral hemithorax, with larger intercostal spaces and the diaphragmatic dome is lowered. The profile of the collapsed lung is sharply outlined (Fig. 64.7).

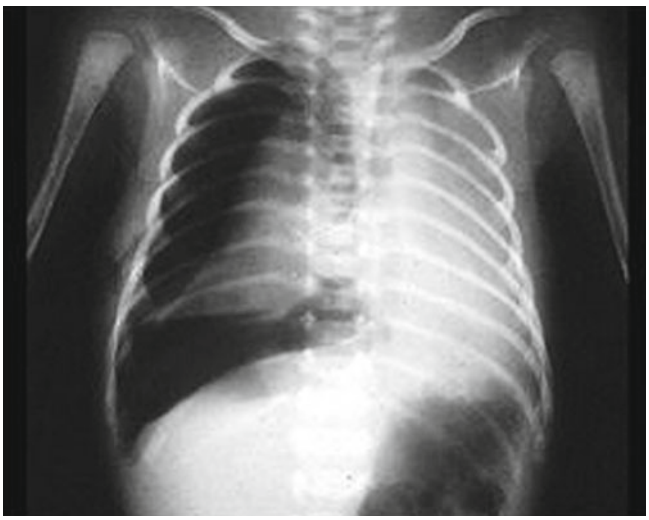

Fig. 64.7 Right tension pneumothorax

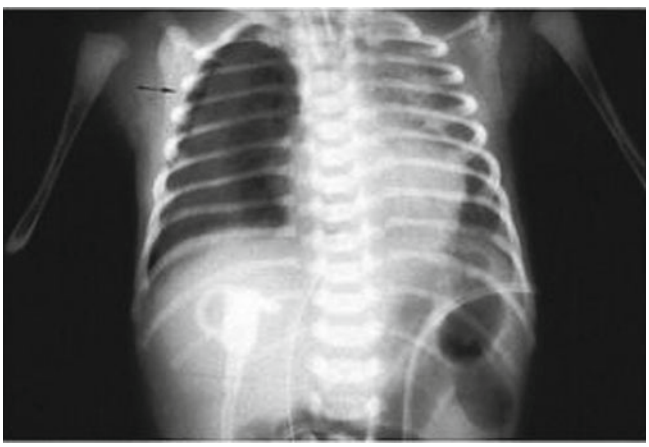

Fig. 64.8 Right antero-medial pneumothorax with a slight contra-lateral shift of the mediastinum

In tension penumothoraces, the contra-lateral shift of the mediastinum is generally considerable. In the most severe cases, the diaphragm is flattened or even concave [15]. Anterior or anteromedial collections of air inside the pleural cavity may easily be missed. The appearances are those of a lung with decreased markings and slight contra-lateral shift of the mediastinum (Fig. 64.8). In patients in whom an antero-medial pneumothorax is suspected, a lateral supine chest X-ray is useful. The thymus gland on the side of the pneumothorax may appear closely adherent to the mediastinum. A large thymus, may give a false impression of a space-occupying mass in the upper mediastinum. Ultrasonography has been shown to be accurate and efficient for the early detection of clinically important pneumothoraces [28].

64.5.3 Differential Diagnosis

- Lobar emphysema.
- Cystic adenomatoid malformation of the lung.
- Congenital diaphragmatic hernia.
- Bronchogenic cyst.
- Esophageal-pleural fistula (Boerhaave syndrome).

The most common complications of pneumothorax in the neonate are: respiratory or cardiac arrest, intraventricular hemorrhage, bronchopleural fistula, hemopneumothorax, and infection.

64.5.4 Therapy and Treatments

64.5.4.1 Asymptomatic Pneumothorax

The asymptomatic pneumothorax does not require any specific treatment other than close observation and may be managed conservatively. In term infants, reabsorption of the free air may be increased by increasing the inspired oxygen concentration (the infant is usually placed in a 100% oxygen hood for 12–24 hours). This treatment should not be used for preterm infants or infants with tension pneumothorax. When mechanical ventilation is used, the pneumothorax should be drained, even if apparently asymptomatic. There may be clinical doubt about whether to drain very small pneumothoraces when the clinical condition of the ventilated patients remains stable, but the risk of an acute tension pneumothorax suggests that intervention is to be preferred.

64.5.4.2 Needle Aspiration

Needle aspiration can be used to treat a symptomatic pneumothorax. It is frequently curative in infants who are not mechanically ventilated and may be a temporizing treatment in infants who are mechanically ventilated.

64.5.4.3 Thoracotomy

Chest tube drainage (thoracotomy) is usually needed for continuous drainage of pneumothoraces that develop in infants receiving positive pressure ventilation because the air leak may persist. In these cases, continuous aspiration with negative pressures of 5–10 cmH_2O may be useful.

Technique of pleural drainage:

a. Select a chest tube of appropriate size. For small infants, 8 Fr chest tubes are adequate. For larger infants, 10 Fr chest tubes are better
b. Locate the fifth intercostal space in the anterior axillary line on the affected side [29]
c. Prepare the site with antibacterial solution
d. Administer analgesic to the infant. Be ready for resuscitation
e. Inject the area with a small amount of 1% lidocaine (1–2 mg/kg)
f. Make a small incision (approximately the size of the tube) along the intercostal space directly over the sixth rib
g. Remove the trochar and insert the tube by blunt dissection
h. Advance the chest tube a few centimetres and check the position radiographically
i. Suture the tube in place. The drain should be left in place for 24–48 hours from the moment the pneumothorax has stopped draining, i.e., when the intermittent air leakage stops. In the case of residual air, attach the tube to a drainage system under low continuous suction (–10 to –20 cmH_20).

Complications of pleural drainage include:

- Infection
- Hemorrhage (laceration of intercostal vessels)
- Lung puncture
- Bronchopleural fistula, which is evidenced by persisting pneumothorax
- Chylothorax (secondary to lesions of the thoracic duct)
- Paralysis of the diaphragm (due to lesions of the phrenic nerve).

64.5.4.4 Intubation

Selective intubation of the contralateral main bronchus should be reserved for cases where there is a bronchopleural fistula.

64.5.5 Prevention

Pneumothorax may be prevented by using ventilation techniques that allow for synchronization between spontaneous and mechanical breaths so as to reduce transpulmonary pressure fluctuations. HFOV may reduce the incidence of pneumothorax in infants with severe pulmonary disease.

64.6 Pneumopericardium

Pneumopericardium is a collection of air in the pericardial space. It frequently occurs in association with pneumomediastinum. It is likely that gas enters the pericardium through a defect in the pericardial sac, probably at the pericardial reflection near the ostia of the pulmonary veins. The incidence has been estimated around 1.3% of term and preterm infants [30]. Pneumopericardium rarely occurs spontaneously [31].

Risk factors for pneumopericardium are:

- Prematurity
- Birth asphyxia requiring resuscitation
- Mechanical ventilation (PIP >32 cmH_2O, MAP >17 cmH_2O)
- Lung disease
- Anatomic predisposition (maldevelopment of the left pleuropericardial membrane).

64.6.1 Etiology and Pathogenesis

Pneumopericardium is frequently associated with a pneumomediastinum and/or pneumothorax since the pathogenic mechanism is the same, i.e., diffusion of air from the pulmonary interstices through the bronchovascular sheaths to the planes of pleural reflection at the level of the large vessels, where air under tension flows directly into the pericardium sac. Mediastinal air collection may spread around the heart because of congenital defects of the pericardium.

64.6.2 Clinical Aspects

Because of the speed at which it occurs, pneumopericardium is usually a dramatic event, causing cardiac tamponade. Symptoms appear when the pressure in the pericardium exceeds the ventricular filling pressure and cardiac output is reduced [31, 32]. Sometimes the presenting signs of pneumopericardium may be confused with those of a tension pneumothorax. The heart sounds are muffled, but a friction rub is occasionally audible. There are low voltages on electrocardiography. The radiological appearances of pneumopericardium are of a hypertransparent image, which outlines the heart entirely, except for where the great vessels emerge (Fig. 64.9).

64.6.3 Therapy and Treatments and Prognosis

Treatment is conservative if asymptomatic. Oxygen administration will help the reabsorption of air in cases with mild respiratory distress [33]. If cardiac tamponade occurs,

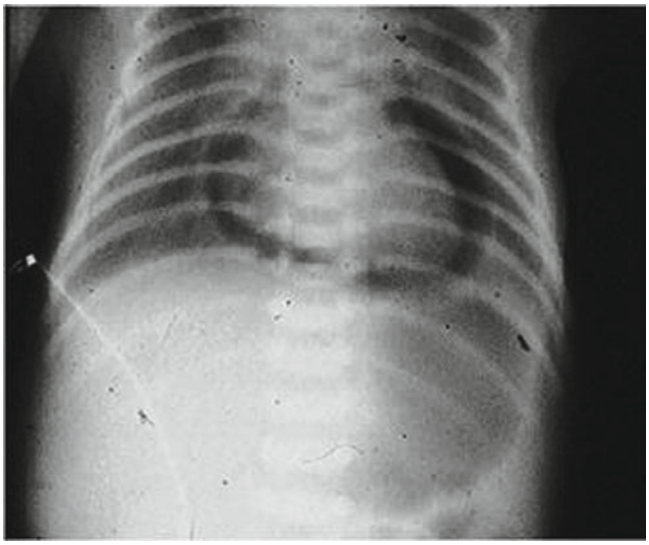

Fig. 64.9 Pneumopericardium: a hypertransparent image that entirely delimits the heart except for the emergence of the great vessels

drainage with a cannula needle will be performed via the subxiphoid route. Relapses are frequent and require the positioning of an in-dwelling drainage tube [34]. Mortality is high, between 70 and 90%.

64.7 Pneumoperitoneum

Pneumoperitoneum is defined as the presence of air within the peritoneal cavity.

64.7.1 Etiology and Pathogenesis

Pneumoperitoneum usually results from perforation of the gut, although in some instances it can be caused by air under pressure dissecting from the chest (pneumomediastinum, bronchopleural fistula) through the diaphragmatic apertures into the peritoneal space [35, 36].

64.7.2 Clinical Aspects, Therapy and Treatments

Pneumoperitoneum may be an incidental finding or may present with abdominal distension or in most severe cases with cardiovascular collapse.

On the posteroanterior radiograph, pneumoperitoneum may be difficult to see if free intaperitoneal gas accumulates between intestinal loops. If gas is present on both sides of the bowel it may outline the bowel wall, rendering it visible as a thin, linear stripe (double wall sign or Rigler's sign). If there is doubt about the diagnosis of pneumoperitoneum, consider a lateral shoot-through abdominal X-ray (with the baby lying on his back and the X-ray film at right angles to the mattress), or a lateral decubitus abdominal X-ray, both of which will demonstrate the liver clearly separate from the abdominal wall. Free abdominal air requires a surgical opinion. Either peritoneal drainage or laparotomy may be required.

64.8 Pulmonary Gas Embolism

The presence of intravascular gas is a rare complication of positive pressure ventilation [37]. This condition is usually fatal and results from direct communication between airway lumen, interstitium and small vascular channels, as demonstrated by barium studies at autopsy [38]. It can be a consequence of air leaks syndromes or lung trauma.

64.8.1 Clinical Aspects, Therapy and Treatments

Affected infants are usually premature and have severe respiratory failure necessitating very high ventilatory pressures. Pulmonary gas embolism causes a catastrophic deterioration of the patient's condition with cardiocirculatory collapse.

The radiographs of thorax and abdomen show pulmonary interstitial emphysema and pulmonary venous air embolism (i.e., air within the heart and many vessels).

Treatment is directed to sustain cardiopulmonary function.

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65

Bronchopulmonary Dysplasia/Chronic Lung Disease

Vineet Bhandari

65.1 Introduction

Bronchopulmonary dysplasia (BPD) is the most common cause of chronic lung disease in infancy [1]. The consensus definition of BPD has been summarized in Table 65.1. The "classic" BPD described by Northway in 1967 has now been replaced by less severe forms of "new" BPD, which are infrequently found in patients >30 weeks of gestation and with birth weights (BW) >1200 grams. Presently, infants who weigh <1250 grams account for 97% of all BPD patients [2]. The incidence of BPD, defined as oxygen need at 36 weeks post-menstrual age (PMA), was 52% (BW 501–750 g), 34% (BW 751–1000 g), 15% (BW 1001–1200g), and 7% (BW 1201–1500 g). Using a physiologic definition based on an oxygen reduction challenge at 36 weeks PMA led to a 10% decrease in the incidence of BPD.

65.2 Pathology

The pathology of "old" BPD (pre-surfactant era) was remarkable for the presence of severe airway injury, inflammation and parenchymal fibrosis. There was marked heterogeneity in lung pathology with severe alveolar septal fibrosis in some areas and presence of normally inflated and/or hyperinflated lung in the adjacent sub-lobule or lobe. Post-surfactant use, the "new" BPD lung revealed a more uniform inflation and less marked fibrosis and absence of small and large airway epithelial metaplasia, smooth muscle hypertrophy and fibrosis. Arrest of acinar development resulting in a decrease in alveolar number and arterial counts with a normal alveolar/arterial ratio was reported in the lungs of BPD patients regardless of surfactant treatment. Abnormalities of distal microvasculature have been linked to arrest/impairment of vascular development. There is controversy about the pulmonary microvasculature being decreased or increased; however, it is undoubtedly dysregulated in BPD [1, 3].

The observed vascular changes include marked angiogenesis proportionate to the growth of the air-exchanging lung parenchyma, abnormal distribution of alveolar capillaries, prominent corner vessels with variable capillary density in adjacent alveoli, or vessels that are more distant from the air surface.

65.3 Etiology and Pathogenesis

Genetic predisposition, baro- and volutrauma from mechanical ventilation in surfactant-deficient premature lungs, pulmonary edema, pre- and postnatal infections, and reactive oxygen species from prolonged oxygen use and high oxygen concentrations, have been associated with the development of BPD (Fig. 65.1).

65.3.1 Genetics

Genetic factors contribute 53–82% susceptibility [4, 5], making BPD a disease occurring secondary to "gene-environmental" factors. In the first study, it was observed that in premature twins (63 monozygotic and 189 dizygotic twin pairs), <32 weeks gestational age (GA), BPD occurred at a rate of 29% in one or more of a twin pair. After controlling for other covariates, 53% ($p = 0.004$) of BPD was explained by genetic factors alone [4]. These results were independently confirmed [5]. Multiple genetic variants have been assessed in the evaluation of carrier states that predispose to the development of BPD [6, 7].

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 65.1 Diagnostic criteria for BPD

	MILD Supplemental O_2 (for 28 days) and	MODERATE Supplemental O_2 (for 28 days) and	SEVERE Supplemental O_2 (for 28 days) and
<32 weeks GA at birth	RA at 36 weeks corrected GA or at discharge	<0.3 FiO_2 at 36 weeks corrected GA or at discharge	≥0.3 FiO_2 +/– positive pressure support at 36 weeks corrected GA or at discharge
≥32 weeks GA at birth	RA by postnatal day 56 or at discharge	<0.3 FiO_2 by postnatal day 56 or at discharge	≥0.3 FiO_2 +/– positive pressure support by postnatal day 56 or at discharge

BPD bronchopulmonary dysplasia, *FiO_2* fraction of inspired oxygen, *GA* gestational age, *RA* room air. From [56], with permission.

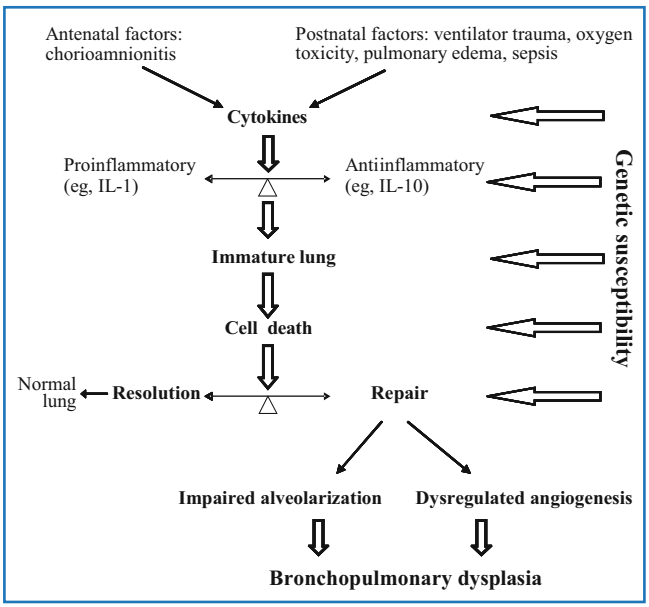

Fig. 65.1 Pathogenesis of "new" BPD. From [56], with permission

65.3.2 Prenatal Infections

Chorioamnionitis may be a risk factor for the development of BPD. Though the pathogenesis is incompletely understood, studies have evaluated cytokines in the amniotic fluid of infants born prematurely. Increased levels of cytokines, predominantly interleukins (IL), IL-1β, IL-6, IL-8, have been noted in many who go on to develop BPD.

Though there is difficulty in correlating clinical and histologic chorioamnionitis, infants born at <28 weeks GA who have evidence of histologic chorioamnionitis have worse pulmonary outcomes than those born at later GA. These infants also had elevated levels of inflammatory cytokines in their blood. Investigators, exploring the relationship between chorioamnionitis and BPD, have indicated that in utero exposure to low level inflammation may induce a pulmonary maturation (as evidenced by decreased rates of respiratory distress syndrome [RDS]); however, there was no decrease in BPD. Other investigators have noted antenatal infection, coupled with postnatal sepsis, and mechanical ventilation increased the rates of BPD in preterm neonates [8].

65.3.3 Lung Immaturity

Intrinsic to the development of BPD is prematurity. Normal lung development occurs in phases that correspond to structural differences. The first phase, the embryonic phase (3–7 weeks PMA) is followed by the pseudoglandular phase (5–17 weeks). Many premature neonates are born during the late canalicular phase (16–26 weeks) of lung development. The saccular phase occurs at 24–38 weeks and accounts for the majority of infants who later develop BPD. The last phase, the alveolar phase, starts from ~32 weeks PMA till about 2 years after birth, with the majority of the alveolarization process occurring 5–6 months after birth. The process of alveolar development can extend longer, possibly up to 8 years. Alveolarization incorporates the processes of "elastogenesis and angiogenesis" with multiple cell-to-cell interactions between fibroblasts, epithelial (type I and II pneumocytes), interstitial, and endothelial cells. Premature birth, with its subsequent medical disorders and their management, alters the postnatal development of lungs, via changes in the molecular signal transduction pathways. Factors that disrupt normal angiogenesis, control of inflammation (i.e., imbalance between "pro"- and "anti-inflammatory" cytokines), and appropriate fibrin deposition or removal are at play in the pathogenesis of BPD.

65.3.4 Hyperoxia

Excessive oxygen induces oxidative stress with production of reactive oxygen species with increased production of inflammatory cytokines from epithelial, endothelial and inflammatory cells. Initial hyperoxic cellular damage induces alveolar or interstitial macrophages to express early cytokines such as IL-1 and tumor necrosis factor (TNF). Chemoattractants are

then induced from multiple cell types in the lung, in turn recruiting other inflammatory cells, such as neutrophils [9]. An immature lung that is exposed to ante- and postnatal environmental factors contributes to the release of a variety of pro- and anti-inflammatory cytokines. An imbalance in these mediators leads to activation of the cellular death pathways in the lung. Thus, direct (via reactive oxygen species) or indirect (via cytokines) damage to the respiratory endothelium and epithelium occurs by inducing necrotic and/or apoptotic cell death [9]. This is followed by healing (resolution of injury) or repair of the lung [10, 11]. The latter process is characterized by impaired alveolarization and dysregulated angiogenesis leading to fewer, larger simplified alveoli and a dysmorphic pulmonary vasculature (Fig. 65.1), the pathologic hallmarks of BPD [1].

65.3.5 Barotrauma and Volutrauma

Mechanical ventilation has long been associated with the development of BPD. Intubated neonates who ultimately develop BPD often have significantly increased levels of "pro-inflammatory" cytokines, possibly from microbial colonization and increased distention of alveolar tissue with subsequent baro- and volutrauma leading to increased inflammation [12]. The association of a patent ductus arteriosus (PDA) and BPD is likely due to the increase in pulmonary circulation and subsequent pulmonary edema. This usually instigates increased ventilatory support and oxygen administration, both being important contributory factors to the development of BPD.

65.3.6 Postnatal Infections

Systemic infections predispose to the development of BPD [8]. The inflammatory response occurring secondary to infection increases release of inflammatory and vasoactive cytokines on an already damaged pulmonary organ, increasing the need for mechanical ventilation. Airway colonization, with increased neutrophilic presence and pro-inflammatory cytokines may also predispose to the development of BPD [12].

65.3.7 Role of Cytokines

The common theme of inflammation noted above is underscored by the role played by cytokines. Cytokines are chemicals produced by virtually all cell types including fibroblasts, white blood cells, endothelial and epithelial cells. They mediate immune, inflammatory and hematopoietic functions in response to various stimuli. In preterm infants, inciting events cause an inflammatory response, either systemically or localized to the pulmonary organ. We have only a partial understanding of how inflammation in the setting of prematurity causes BPD. Soon after birth, upon initiation of oxygen exposure and/or mechanical ventilation, there is an influx of neutrophils and macrophages into pulmonary interstitium. Neutrophils adhere to the endothelium, with interaction between these two cell types occurring via adhesion molecules (eg., selectins and integrins). This allows for extravasation of neutrophils and macrophages towards the specific areas of injury. These inflammatory cells produce cytokines and other signaling molecules for augmentation of the inflammatory response in an attempt to mitigate the damage of the inciting insult. There is subsequent disruption of the alveolar capillary unit and pulmonary tissue integrity. The imperfect attempt at repair, differentiation and growth of these tissues act in concert with the inflammation to produce the biochemical profile, signs and symptoms we see in patients with, and those at risk, for BPD [12, 13].

Many studies have been conducted to evaluate the presence of cytokines in the amniotic fluid, cord blood, plasma and tracheal secretions of preterm neonates at risk or who have BPD in an effort to determine a biochemical profile of the condition. Many of the pulmonary biomarkers relate to the possible disruption in the mechanisms described above. A multitude of biomarkers detected in tracheal aspirates (TA), blood and urine have been proposed for early identification of infants who subsequently develop BPD.

65.4 Biomarkers in BPD

Though there are many clinical associations indicative of an increased risk for the eventual development of BPD, currently no one factor or marker exists that uniformly and accurately predicts its development. The inciting events that predispose to development of BPD likely occur fairly soon after birth, and we are limited by a lack of ability to distinguish very early on, those neonates who will likely go on to develop this chronic disorder. A majority of the biomarkers discussed below are likely abnormal due to damage that has already occurred. Some biomarkers are capable of being perceived as either pro- or anti-inflammatory depending on their concentration in relation to their physiologic role. Given the proximity to the lung, the primary focus of most investigators has been to access pulmonary secretions to evaluate putative biomarkers for BPD.

The initial TA studies focused on the cytology of the secretions. The exfoliation of dysplastic, metaplastic bronchial cells was found to be 95% specific and 71% sensitive for the subsequent development of BPD. Categories of pulmonary biomarkers that are deranged in BPD include IL and other chemoattractant molecules, markers indicative of abnormal angiogenesis, those signifying abnormal fibrin deposition or

Table 65.2 Use of tracheal aspirate analytes as biomarkers for BPD [14]

Analyte	Total no. of infants*	Association with BPD
Neutrophil counts	30; 20	Increased
Fibronectin	32	Increased
Elastase	20; 65	Increased
Lactoferrin	36	Decreased
Lysozyme	36	Decreased
PAI-1	37	Increased
Trypsin-2	32	Increased
Cathepsin-K	13	Decreased
sICAM-1	20;15	Increased
CCSP	45	Present
IL-1β	28; 20	Increased
IL-6	28; 20; 75; 65; 34	Increased
IL-8	20; 65; 34; 35; 31	Increased
TNF-α	28	Increased
MCP-1	35; 56	Increased
MCP-2	56	Increased
MCP-3	56	Increased
ET-1	34; 29	Increased; no association
TGF-β	30	Increased
FGF-2	29	Increased
KGF	91	Decreased
HGF	22	Decreased
VEGF	29; 44	No association; decreased
PTHrP	40	Decreased
Angiopoietin 2	14; 60	Increased
MIF	26	Decreased

* Per study cited in [14].
PAI plasminogen activator inhibitor, *sICAM* soluble intracellular adhesion molecule, *CCSP* Clara cell secretory protein, *IL* interleukin, *TNF* tumor necrosis factor, *MCP* monocyte chemoattractant protein, *ET* endothelin, *TGF* transforming growth factor, *FGF* fibroblast growth factor, *KGF* keratinocyte growth factor, *HGF* hepatocyte growth factor, *VEGF* vascular endothelial growth factor, *PTHrP* parathyroid hormone-related protein, *MIF* macrophage migration inhibition factor.

degradation, markers of oxidative damage, and peptide growth factors. While a study has shown that TA specimens may be suitable substitutes for bronchoalveolar lavage samples in preterm infants, there has been some controversy about whether measurements of the markers in the TA are valid without normalization. While some have advocated use of total protein, secretory component of IgA, and serum urea, others have espoused that no normalization is required.

Table 65.2 summarizes the results of various TA analytes, focusing more on recent reports that would be more reflective of the "new" BPD, usually obtained in the first week of life, from babies at risk of developing BPD. More details are available from a recent review [14].

65.4.1 Interleukins (IL)

IL are chemicals made by one cell type that act on other inflammatory cells. They may induce growth, differentiation and proliferation of other leukocytes. They may also act as chemoattractants for recruitment of other cell types (immune and non-immune cells) to a site of injury, and may exert pro- or anti-inflammatory effects.

IL-1β, 6, 8, 16 IL-1β and 6 are particularly active in the acute phase response to injury, while IL-8 is a potent chemotactic agent for recruitment of neutrophils. These cytokines are typically viewed as pro-inflammatory, and have been shown to be elevated in the TA very early in the respiratory course of the preterm population that ultimately develops BPD. IL-16 acts as a chemoattractant for CD4+ T lymphocytes, monocytes and eosinophils. Levels of IL-16 correlated significantly with neutrophil counts in the TA and subsequent development of BPD.

IL-10, 4, 13 These cytokines usually act in an anti-inflammatory capacity, and are responsible for the production, differentiation and proliferation of B-cells and macrophages. It has been suggested that decreased levels of anti-inflammatory cytokines would predispose to the development of BPD given the role that inflammation likely has in this disorder. However, there are discrepant findings regarding the levels of anti-inflammatory cytokines in the TA of preterm infants at risk for BPD.

IL-10 IL-10 was found to be decreased in TA samples taken in the first seven days of life in those infants who developed BPD. Other investigators found detectable levels of IL-10 in TA of preterm ventilated infants, but could not correlate the levels with development of BPD. When lipopolysaccharide (LPS), a potent inducer of inflammation was used to induce production of IL-10 from lung inflammatory cells (obtained from premature infants) there was a decreased ability to induce IL-10 in those infants who went on to develop BPD. A majority of preterm infants (97%) unable to constitutively express the IL-10 gene developed BPD.

IL-4, 13 IL-4 is produced by T lymphocytes and alveolar macrophages. Levels of these potentially anti-inflammatory cytokines (IL-4, 13) could not be significantly correlated with the development of BPD.

65.4.2 Angiopoietin-2

Angiopoietin-2 is an angiogenic growth factor that destabilizes blood vessels, enhances vascular leak and induces epithelial cell necrosis in hyperoxic conditions. Angiopoietin-2 concentration was observed to be increased in infants with BPD and/or death in the first week of life, when compared to those infants with RDS who recovered [15, 16]. Furthermore, the levels decreased after exposure to dexamethasone [16].

65.4.3 Cathepsin K

Cathepsin K is a cysteine protease, which degrades existing extracelluar matrix and regulates the release of matrix proteins from fibroblasts. In one study, infants who developed BPD had declining cathepsin K levels by day 13, when compared to neonates who did not develop BPD.

65.4.4 Chemokines

There are 4 families of chemokines (chemotactic cytokines, CC); they play a role in regulating inflammation by recruiting inflammatory cells to the area of injury. The CC family consists of monocyte chemoattractant proteins (MCP) 1, 1α, 1β, 2 and 3. These chemokines were measured in a study to assess the role of CCs in acute lung injury in the preterm, ventilated infant over the first 21 days of life. MCP-1, 1α, 1β, 2 and 3 were all increased in those infants developing BPD. However, only MCP-1, 2 and 3 were statistically significant, with MCP-3 being the most significant [17].

65.4.5 Clara Cell Secretory Protein (CCSP)

CCSP is produced by Clara cells, which are non-ciliated epithelial cells lining the respiratory and terminal bronchioles. This protein may act to modulate acute pulmonary inflammatory processes. In preterm infants with TA samples taken up to 2 weeks after birth, CCSP increased with maturity, and in those neonates who had evidence of infection. In another study, the results indicated that lower levels of CCSP predisposed to development of BPD.

65.4.6 3-Chlorotyrosine

3-Chlorotyrosine is a biomarker of the neutrophil oxidant, hypochlorous acid, which is utilized in the inflammatory process. The only source is from neutrophils and monocytes which release myeloperoxidase to catalyze oxidation of chloride by hydrogen peroxide to give hypochlorous acid. In support of data that neutrophilic infiltration and subsequent oxidative injury is closely related to the development of BPD, investigators assessed levels of 3-chlorotyrosine, and found that an increase in their concentration correlated with development of BPD.

65.4.7 Fibronectin

An extracellular matrix component, fibronectin is important in maintaining the integrity of pulmonary tissues and microvasculature. Researchers have found a higher fibronectin level in those infants who developed BPD.

65.4.8 Lactoferrin and Lysozyme

Lactoferrin is said to be a marker of inflammation by regulating granulocyte and macrophage proliferation, and may also have some antioxidant properties. Lysozyme is a bactericidal protein that degrades the walls of susceptible bacteria. Both are hypothesized to be released from the serous cells of the submucous glands of the respiratory tract. Both lactoferrin and lysozyme when measured in the first 3 days of life were decreased in patients with BPD as compared to those without BPD, with the decrease in lysozyme being significant.

65.4.9 Macrophage Migration Inhibitory Factor (MIF)

MIF is an upstream regulator of the innate immune response. It has been implicated in the pathogenesis of a number of inflammatory disorders including sepsis, acute RDS (in adults), asthma, and inflammatory/autoimmune diseases. MIF was quantified in TA obtained during the first 2 days of life in a cohort of neonates with RDS. There was a reduction in the concentration of MIF in the lungs of those infants who went on to develop BPD [18].

65.4.10 Malondialdehyde

Malondialdehyde is marker of oxidative damage, possibly from oxygen radicals produced under hyperoxic conditions, or the respiratory burst from inflammatory cells present in the pulmonary organ. In one study of ventilated infants, the pulmonary concentrations were noted to be elevated, but weakly correlated with the development of BPD.

65.4.11 Matrix Metalloproteinases (MMPs)

MMPs are a family of endoproteinases that aid in the remodeling and degradation of extracellular matrix and basement membranes. They are secreted in inactive form, activated in extracellular spaces and cell surfaces by oxidants and serine proteinases. They also have the capacity to activate each other. The development of BPD is partially characterized by disordered pulmonary repair after inflammation. Patients who went on to develop BPD had increased levels of tracheal MMP-8, with decreased levels of its inhibitor, tissue inhibitor of metalloproteinases (TIMP) [19].

65.4.12 Nuclear Factor-kappa B (NF- κB)

NF-κB is a transcription factor activated by cellular stress, subsequently promoting expression of multiple genes including pro-inflammatory cytokines. TA levels of NF-κB were significantly elevated in the infants who developed BPD or died. However, this difference did not persist when GA was taken into account.

65.4.13 Parathyroid Hormone-Related Protein (PTHrP)

PTHrP is secreted by type II pneumocytes and plays a role in normal alveolar growth and development. Its signaling was shown to be down-regulated in alveolar over-distention and under hypoxic conditions. Infants developing BPD had significantly lower levels of PTHrP in the first week of life, when compared to those who did not [20].

65.4.14 Plasminogen Activator Inhibitor - (PAI-1)

PAI-1 is a regulator of fibrinolysis. It was hypothesized that inhibition of fibrinolysis may play a role in the development of BPD. Infants who developed BPD had significantly higher levels of PAI-1 than non-BPD patients.

65.4.15 Pulmonary Trypsin-2

Trypsin-2 is a serine protease expressed in a variety of human epithelial cell types, including in the lung. It can directly attack extracellular protein matrix, basement membrane proteins, and can activate MMPs and initiate protease cascades, causing tissue destruction. It was hypothesized that when trypsin is ineffectively inhibited by its natural inhibitor, tumor-associated trypsin inhibitor (TATI), it may play a role in the development of BPD. Results in TA samples from preterm infants indicated that there was significantly higher trypsin-2 to TATI ratio during first 2 weeks in those infants developing BPD.

65.4.16 Soluble Intercellular Adhesion Molecule-1 (ICAM-1)

ICAM-1 plays a role in early inflammation and is the ligand for lymphocyte function-associated antigen 1 (LFA-1). ICAM-1 is expressed by lymphocytes, eosinophils, mast, endothelial and bronchial epithelial cells. Infants who developed BPD had significantly higher levels of soluble ICAM-1 in TA at 6-14 days of age.

65.4.17 Transforming Growth Factor–β1 (TGF-β1)

TGF–ß1 is produced mostly by alveolar macrophages, and acts on fibroblasts to increase the transcription of fibronectin and procollagen. It also inhibits synthesis of proteases and increases the synthesis of anti-proteases, thus resulting in a net increase in fibrosis. Infants who developed BPD had significantly increased levels of TGF-β1 in TA.

65.4.18 Tumor Necrosis Factor–α (TNF-α)

TNF-α is a pro-inflammatory cytokine that induces cell death, and enhances expression of other cytokines. In a study of premature infants, TNF-α was noted to be increased early in the patients who developed BPD.

65.4.19 Peptide Growth Factors

Peptide growth factors are important for normal lung development, maturation and repair. This group includes endothelin-1 (ET-1), fibroblast growth factor-2 (FGF-2), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF).

ET-1 ET-1 is a potent endothelium-derived vaso- and broncho-constricting factor, produced in virtually all tissues, though its largest concentration is in the lung. Under the influence of cytokines (IL-1β, IL-6, IL-8), production of ET can be induced in macrophages and monocytes. ET-1 has also been hypothesized to increase the production of oxygen radicals from alveolar macrophages. In preterm infants with RDS, TA concentrations of ET-1 in the first week of life were significantly elevated in infants developing BPD [21]. However, this was not confirmed by others [22].

FGF-2 FGF plays a role in angiogenesis, by stimulating endothelial cell proliferation, degrading extracellular matrix and appears to interact with VEGF. There was a significant elevation in the levels of FGF-2 in the TA in the BPD/death population on day 1 of life [22].

KGF A member of the FGF family, KGF regulates proliferation of alveolar epithelial cells, and enhances synthesis of surfactant and accelerates wound closure in airway epithelium. It was noted that in premature infants who developed BPD, KGF was lower than in those who did not develop it.

Pulmonary HGF HGF in fetal rat lungs stimulates branching of both alveolar and bronchial epithelia, and contributes to growth, maturation and maintenance of tissue homeostasis.

It has been suggested that HGF also mediates repair of type II pneumocytes after acute lung injury. Infants who developed BPD had significantly lower levels of HGF in the first 2 weeks of life, when compared to non-BPD counterparts.

VEGF VEGF promotes endothelial cell growth and remodeling. In the pulmonary system it appears to be essential for the appropriate development of alveolar tissue; in rats its antagonism has been shown to result in dramatically impaired alveolarization [23]. In heavily vascularized tissues, such as the lung, VEGF exists in high concentrations. During hyperoxic episodes when damage to the microvasculature occurs, VEGF plays a role in the remodeling process, and its levels are increased, sometimes disproportionately. In preterm infants with RDS, infants developing BPD had significantly lower levels of TA VEGF closer to one week of life [14]. Conversely, no difference in TA VEGF levels were noted between BPD and no BPD infants in another study [22]. In a recent study, a phasic pattern of VEGF concentrations was noted in infants who go on to develop BPD. These infants had an initial spike over the first 12 hours of postnatal life, followed by a decrease over the next few days and then a subsequent significant increase [24].

65.4.20 Other Biomarkers

Given the complexity of the pathogenesis of BPD, investigators have also attempted to use the relative concentration of TA markers to predict BPD. Infants who developed BPD had higher IL-1β concentrations and IL-1β/IL-6 ratios. Premature infants who developed BPD had higher levels of IL-1 receptor antagonist (IL-1RA) in their airways on the first day of life. IL-1β also increased significantly for BPD patients early, both compared to non-BPD patients, and also within the BPD group. While the early (day 1) IL-1 antagonist/agonist molar balance offered protection, by days 5 and 7, a threshold for IL-1RA in the presence of increasing IL-1β expression favored pro-inflammation in the BPD group. Others have demonstrated a sustained increase of TGFβ1 levels in TA from preterm infants who developed BPD, combined with an absent or irregular secretion of IL-4, IL-10 and IL-12. Babies who developed BPD or died had lower IL-6/VEGF ratios in the TA, than those who did not have such an adverse outcome [14]. In other studies, BPD infants had significantly lower TIMP-1 levels with a higher MMP-9/TIMP-1 ratio during the first 2 weeks of life and low TIMP-2 and MMP-2 levels during the first 3 days of life compared with no BPD infants [25].

Cord blood (and TA) soluble E-selectin has been noted to be increased in infants who developed BPD. Increased serum levels of soluble E-selectin were associated with the development of BPD. In another study, low levels of soluble L-selectin and increasing levels of soluble E-selectin were reported to be potential risk factors for BPD. The cord blood placental growth factor concentrations were noted to be increased in infants developing BPD. Serum C-IV (an antigen marker of type IV collagen, a component of basement membranes) levels were elevated in the first week of life in infants subsequently diagnosed with BPD. In another study of serial measurements of the C-terminal fragment of type I procollagen, levels at week 4 were significantly lower in infants who subsequently developed BPD. Patients with both the old and new categories of BPD had elevated serum levels of TGF- β1, though these were not statistically significant [26]. Other blood markers include measures of eosinophilic activation (eosinophilic cationic protein and the cellular surface antigen CD9), which were increased in BPD. The plasma KL-6 (a circulating high molecular weight mucinous glycoprotein, which is the extracellular soluble region of MUC-1 mucin, possessing an undefined sialylated carbohydrate chain that is recognized by KL-6 antibody) was noted to be increased in patients with moderate/severe BPD. The plasma levels of 8-iso-prostaglandin F2alpha (a stable metabolite of F2- isoprostanes, a marker for oxidant injury) have been noted to be increased in infants who developed BPD/died. In another study, however, urinary levels of the same metabolite were not correlated with BPD.

Older infants with established BPD had higher levels of urinary leukotriene E 4 when compared to healthy infants of the same age; however, it was not found to be an early marker of BPD. Elevated urinary elevated bombesin-like peptide levels in infants at 1–4 days after birth were associated with a 10-fold increased risk of developing BPD [27]. Given the importance of vascular development in the pathogenesis of BPD, investigators have postulated evaluating the vascularity of the lower gingival and vestibular oral mucosa as a marker for BPD. Infants with BPD showed a significantly lower blood vessel area as well as a higher vascular network complexity than control subjects.

A majority of these studies comprise of a small number of babies. Appropriately so, most attempts to identify biomarkers have been to access the pulmonary compartment by collecting TA. However, TA have been collected at a variable number of time points, measurements usually done of single markers, with or without normalization and with variable definitions of BPD (oxygen requirement for 28 days or at 36 weeks PMA, with or without radiographic criteria). Few have been replicated in different cohorts. Those with consistent results include: elastase, IL-1β, IL-6, MCP-1, ET-1 and angiopoietin 2 (Table 65.2). VEGF has been replicated but showed variable results (Table 65.2). Most of the above comments are also true of the blood and urinary markers. Not surprisingly, none of the aforementioned markers for BPD are in general clinical use.

The majority of the above biomarkers show only an association with BPD. In order to prove a causal relationship, they need to be studied in developmentally-appropriate animal models and human lungs with BPD. Some progress in this direction has been made with the following: elastin [28], cathepsin-S [29], HGF [30], KGF [31], IL-1β [32], IL-6 [33], MCP-1 [34], TGF-β1 [35], VEGF [36], bombesin-like peptide [37] and angiopoietin-2 [15] but additional work is needed.

65.5 Clinical Aspects

65.5.1 Clinical Diagnosis

The classical presentation is a history of a premature infant with RDS who has been intubated and mechanically ventilated and exposed to supplemental oxygen for the first week of life or longer. Usually these infants experience bouts of postnatal sepsis and have inadequate nutritional intake. Over time, these infants fulfill the diagnostic criteria for BPD, as defined earlier.

In the early phase of BPD (up to the first postnatal week of life), the clinical presentation may be indistinguishable from an infant with RDS. Such infants may require intubation and surfactant administration. Occasionally, premature infants may require minimal respiratory support with nasal continuous positive airway pressure (NCPAP) or nasal intermittent positive pressure ventilation (NIPPV), with a supplemental oxygen requirement of <40%. In the evolving phase of BPD (after 1 week of life to 36 weeks PMA), such infants have not been able to be successfully extubated or have been re-intubated. These infants usually require escalation of ventilatory support and higher concentrations of supplemental oxygen. Signs and symptoms of established BPD (after 36 weeks PMA) include tachypnea, dyspnea, and chronic or intermittent crackles and wheezing. In children with subglottic stenosis secondary to prolonged intubation, there may be presence of a stridor on exam. Noisy breathing exacerbated with high airflow activities such as feeding and agitation may be heard in infants with tracheomalacia and bronchomalacia. BPD spells are episodes of tracheal obstruction associated with episodes of cyanosis and oxygen desaturations and bradycardia, usually occurring secondary to agitation, and are thought to be related to tracheomalacia and bronchomalacia. Cardiac complications of severe BPD include pulmonary hypertension and cor pulmonale. Poor growth may be related to undiagnosed hypoxia, cardiac disease, gastroesophageal reflux and swallow dysfunction or recurrent aspiration.

65.5.2 Radiological Findings

In the early phase of BPD, the chest X-ray shows RDS or in some case, minimal respiratory disease. In the evolving phase, the lung fields become more hazy and dense suggesting pulmonary edema, atelectasis or infiltrates. Recently there has been a change in the radiographic findings associated with established BPD. This change has likely been brought about by the younger GA of surviving infants, widespread use of surfactant, and less aggressive respiratory management strategies. Traditionally, in the pre-surfactant era, BPD was represented on radiographic imaging by emphysematous areas with other portions of the lung displaying volume loss. This correlated with the pathology of a pulmonary specimen that would also exhibit signs of increased inflammation, fibrosis and small airway disease. Over the clinical course of evolving "new" BPD, the radiological signs are fairly nonspecific and the lung fields appear hazy and dense secondary to pulmonary edema and atelectasis (Fig. 65.2). Once the "new" BPD is well-established, the appearance of the lungs progresses to hyperinflation and cystic areas, but without the large cysts typical of the older form of BPD [12, 38].

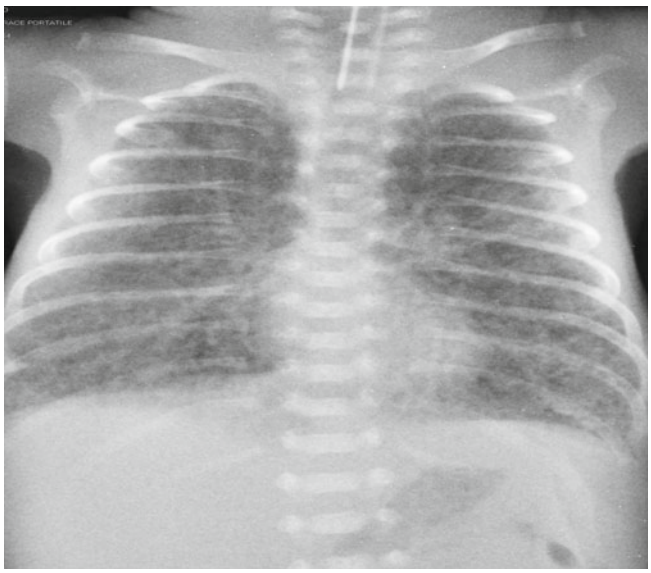

Fig. 65.2 Chest X-ray of an infant with established BPD

65.6 Differential Diagnoses

65.6.1 Aspiration Pneumonia

In the early phase of BPD, respiratory distress can be secondary to aspiration of amniotic fluid or maternal blood during delivery. The radiological picture may show localized lobar infiltrates or an interstitial pattern. With supportive care that usually includes supplemental oxygen with or without mechanical ventilation, intravenous fluids and antibiotics, such infants show significant clinical improvement within the first week of life.

65.6.2 Congenital Heart Disease

Infants with cyanotic congenital heart disease will show evidence of hypoxemia, not responsive to supplemental oxygen. In addition, there may be signs suggestive of a cardiovascular etiology (hypotension, heart murmurs, heart rhythm disturbances). Chest X-rays may be helpful in terms of the size and shape of the cardiac silhouette. Cardiac echocardiography will be diagnostic.

Table 65.3 Differential diagnoses of BPD

- Aspiration pneumonia
- Congenital heart disease
- Infectious pneumonia
- Interstitial lung disease
- Pulmonary lymphangiectasia
- Wilson-Mikity syndrome

65.6.3 Infectious Pneumonia

These are mostly viral or bacterial in the immediate newborn period. There may be maternal history (for example, chorioamnionitis) that may predispose an infant to an infectious pneumonia. Chest X-rays show lobar or perihilar infiltrates. Neonates usually improve with supportive care (as mentioned above) within the first week of life.

65.6.4 Interstitial Lung Disease

In the neonatal period, genetic and developmental disorders leading to interstitial lung disease are most likely to be considered in the differential diagnoses of BPD. These include specific conditions that affect surfactant function i.e., mutations in surfactant proteins –B, -C and ABCA3. Confirmation of diagnoses may require lung biopsy and genetic testing.

65.6.5 Pulmonary Lymphangiectasia

Pumonary lymphangiesctasia is a rare developmental disorder that results in dilated and obstructed lymphatics. Confirmation of the diagnosis requires a lung biopsy. There is an association with Noonan and Turner syndromes.

65.6.6 Wilson-Mikity Syndrome

There is significant overlap between Wilson-Mikity syndrome and the premature infants with BPD who do not have significant respiratory disease at birth (*vide supra*). Infants with Wilson-Mikity syndrome do not develop RDS. The exact etiology is unknown. Proposed predisposing factors include air trapping, fluid overload secondary to chronic PDA, recurrent aspiration, infection, rickets, and surfactant deficiency. Clinical features are characterized by features of respiratory distress. This syndrome is commonly seen between 1 and 2 months of age, and most cases slowly resolve. Chest radiographic findings are normal in the first week, but later they are similar to those of BPD, with hyperinflation, streaky infiltrates, and cystic changes. Radiographic changes persist for a few months to years after clinical findings resolve. It is perhaps a genetic variant of the "new" BPD.

65.7 Prognosis

This is primarily dependent on pulmonary and neurodevelopmental outcomes.

65.7.1 Pulmonary Outcomes

65.7.1.1 Morbidity

There is significant pulmonary morbidity associated with BPD. Compared to non-BPD, infants with BPD have higher rates of rehospitalizations (up to 50%) in the first year, and 36% in the second year of life. Reasons for rehospitalization included reactive airway disease, pneumonia, respiratory syncytial virus (RSV) infection and worsening BPD [39]. Although recurrent respiratory symptoms needing hospitalization decrease over time, this increase in respiratory symptoms persists beyond the first 2 years of life into preschool years, adolescence and early adulthood [39]. It is unclear whether it is the severity of BPD or prematurity per se that influences the persistence and severity of symptoms.

65.7.1.2 Radiological Findings

In children with a history of BPD, abnormal chest X-rays with subtle radiological abnormalities have been noted later in adolescence and adulthood [39]. The chest radiograph is relatively insensitive to the structural changes present in the BPD lung. In contrast, high resolution computed tomography (CT) is much more sensitive for visualizing radiological abnormalities in patients with BPD. Investigators have reported that there is little difference in the radiological appearance of "old" versus "new" BPD, with the absence of bronchial involvement being the only striking difference. A positive correlation between abnormal radiographic findings and pulmonary function has been reported, with severity of the clinical course being associated with extensive CT scan abnormalities [39]. A new chest CT scan scoring system for clinical assessment of patients with BPD has been proposed which correlated well with clinical scores at 36 weeks PMA and duration of oxygen dependence.

65.7.1.3 Pulmonary Function

Despite many advances in neonatal care, patients with BPD continue to have significant impairment in lung function and their lung function may continue to deteriorate into late adolescence [40]. Fortunately, this impaired lung function typically does not interfere with the patient's ability to participate in daily activities, including exercise. Persistent airway dysfunction likely reflects severity of neonatal disease. Although there is a plethora of literature describing abnormal lung function in

patients with BPD at all ages from infancy to early adulthood, there are only a few studies correlating the extent of pulmonary function impairment with various therapies used. An inverse relationship has been reported between FEV1 at school age and duration of supplemental oxygen. V′max25, a measure of flow through small airways, was reduced at school age in inverse proportion to an oxygen score that quantified neonatal supplemental oxygen exposure. Conversely, others have reported poor correlation between duration of mechanical ventilation and oxygen supplementation and forced expiratory flow (FEF) in infants with BPD [39]. BPD infants had low FEF measurements at 6 and 12 months who were treated initially with either high frequency oscillatory ventilation (HFOV) or conventional mechanical ventilation (CMV) compared with published reference values [41]. Infants treated initially with HFOV had significantly better lung function at 12 months than those treated with CMV [41], suggesting that early HFOV in combination with surfactant treatment could lessen the neonatal lung injury that leads to BPD [41]. It is not known if this difference persists into adulthood. Others have found no significant difference in the degree of mild obstructive lung disease as a function of mode of ventilation at 8-9 years of age. This suggests that other factors influence long-term pulmonary outcome [39]. Other studies have noted a correlation in the V′max FRC (forced residual capacity), FEV1 and FEF 25-75% measured at 2 years and school age [39].

Large airways can also be affected in BPD. Prolonged endotracheal intubation and mechanical ventilation result in tracheomalacia and bronchomalacia, which are associated with increased airway compliance. Other problems commonly encountered are subglottic stenosis, airway granulomas and pseudopolyps that may require surgical intervention [39].

Most studies show no reduction in exercise capacity in children with BPD when compared to children that were healthy term infants or preterm babies without lung disease [39]. Decreased gas transfer and oxyhemoglobin saturations during exercise in school aged children (6–9 years) has been reported in those with a history of BPD when compared with full-term and preterm children without BPD. Others have shown a decrease in maximal exercise in capacity in BPD survivors aged 6–12 years when compared to healthy controls. Exercise tolerance continues be relatively unchanged in young adulthood. Higher airway obstruction and CO diffusing capacity and decreased exercise capacity but normal mean lung function in former premature babies at young adulthood (mean age 19 years), when compared to controls, has been reported. This suggests that the impaired exercise tolerance in these young adults was not related to pulmonary function but perhaps poor physical fitness [39].

Many changes occur in pulmonary function with growth and development. While lung mechanics and some volumes may normalize over time, small airway dysfunction persists. Whether the pulmonary dysfunction in these patients will predispose them to obstructive lung disease as older adults remains to be seen.

65.7.2 Neurodevelopmental Outcomes

BPD children have a greater fine and gross motor skill impairment as well as cognitive function and language delay compared to infants without BPD. Children with severe BPD had worse outcomes and required more interventions at age 8 years than did children with mild or moderate BPD [42].

Most studies of BPD have been cross-sectional in nature and the independent effect of BPD is difficult to assess, given the high likelihood of the presence of additional medical complications in these infants. While preterm infants have an increased risk of neurodevelopmental impairment, BPD is an additional risk factor [43]. BPD does not appear to be associated with a specific neuropsychological, but rather a global impairment [43]. Importantly, the spectrum of neurodevelopmental impairment appears to correlate well with the BPD disease severity [44, 45]. As with pulmonary outcomes, ongoing data will be needed to assess the neurodevelopmental outcomes in infants with “new” BPD.

65.8 Therapy

The current status of the various therapeutic approaches in the 3 (early, evolving and established) phases of BPD is shown in Tables 65.4A-C. The robustness of the evidence has been classified based on the type of clinical studies using the system developed by the U.S. Preventive Services Task Force:

- Level I: Evidence obtained from at least one properly designed randomized controlled trial.
- Level II-1: Evidence obtained from well-designed controlled trials without randomization.
- Level II-2: Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one center or research group.
- Level II-3: Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled trials might also be regarded as this type of evidence.
- Level III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

The recommendation for use in the clinical setting is also based on the guidelines developed by the U.S. Preventive Services Task Force:

- Level A: Good scientific evidence suggests that the benefits substantially outweigh the potential risks.
- Level B: At least fair scientific evidence suggests that the benefits outweigh the potential risks.
- Level C: At least fair scientific evidence suggests that there are benefits provided, but the balance between benefits and risks are too close for making general recommendations.
- Level D: At least fair scientific evidence suggests that the risks outweigh potential benefits.

Table 65.4A Management of BPD: Early phase (up to 1 postnatal week)

Therapeutic intervention	Current status	Level of evidence	Level of recommendation
Oxygen supplementation	A wide variation in the acceptable oxygen saturation levels exists across centers, but generally <95% [57]; usually between 85–93%	I	A
Ventilatory strategy	Avoid intubation. If intubated, give "early" surfactant [58]	I	A
	Use short inspiratory times (0.24–0.4s) [59], rapid rates (40–60/min), low PIP (14–20 cmH_2O), moderate PEEP (4–6 cmH_2O), low tidal volume (3–6 mL/kg) [60]	I III III III III	A B B B B
	Extubate early to SNIPPV/NCPAP [61]	I	A
	Blood gas targets: pH 7.25–7.35 PaO_2 40–60 mmHg $PaCO_2$ 45–55 mmHg [62]	III III I	B B C
	High frequency ventilation for "rescue", if conventional ventilation fails [57]	I	A
Methylxanthines	Improves successful extubation rate [63]	I	A
	Decreases BPD [64]	I	A
Vitamin A	If considering use, dose is 5000 IU administered intramuscularly 3 times per week for 4 weeks. 1 additional infant survived without BPD for every 14 to 15 infants who received vitamin A [65, 66]	I	A
Fluids	Restrictive fluid intake may decrease BPD [67, 68]	II-2	B
Nutrition	Provide increased energy intake [69]	I	B

PIP peak inspiratory pressure, *PEEP* positive end expiratory pressure, *SNIPPV* synchronized nasal intermittent positive pressure ventilation, *NCPAP* nasal continuous positive airway pressure. From [56] with permission.

Table 65.4B Management of BPD: Evolving phase (>1 postnatal week to 36 weeks PMA)

Therapeutic intervention	Current status	Level of evidence	Level of recommendation
Oxygen supplementation	Same as in Table 65.4A	I	A
Ventilatory strategy	Avoid endotracheal tube ventilation. Maximize non-invasive ventilation (SNIPPV/NCPAP) for respiratory support [61]	I	A
	Blood gas targets: pH 7.25–7.35 PaO_2 50–70 mmHg $PaCO_2$ 50–60 mmHg [60]	III III III	B B B
Methylxanthines	Same as in Table 65.4A	I	A
Vitamin A	Same as in Table 65.4A. If using, continue for 4 postnatal weeks	I	A
Steroids	Dexamethasone effective in weaning off mechanical ventilation when used "moderately early" and "delayed" [70, 71]	I	A
	Increased incidence of neurological sequelae with early use (<96 hours) [72]	I	D
Diuretics	Furosemide: may use daily or every other day with transient improvement in lung function [73]	I	B
	Spironolactone and Thiazides: chronic therapy improves lung function, decreases oxygen requirements [73]	I	B
Nutrition	Same as in Table 65.4A	I	B

PMA post-menstrual age, *SNIPPV* synchronized nasal intermittent positive pressure ventilation, *NCPAP* nasal continuous positive airway pressure. From [56] with permission.

Table 65.4C Management of BPD: Established phase (>36 weeks PMA)

Therapeutic Intervention	Current Status	Level of Evidence	Level of Recommendation
Oxygen supplementation	For prevention of pulmonary hypertension and cor-pulmonale. A wide variation in the acceptable oxygen saturation levels exists across centers, but generally ~95% [57, 74]	III	C
Ventilatory strategy	Blood gas targets: pH 7.25-7.35 PaO_2 50-70 mmHg $PaCO_2$ 50-65 mmHg [60]	III	B
Steroids	Oral prednisolone may be helpful in weaning oxygen [75]	II-2	C
Diuretics	Chronic therapy as in Table 65.4B	I	B
Beta agonists	Transient relief: increased compliance and reduced pulmonary resistance [73]	I	C
	No significant effect on incidence or severity of BPD [76]	I	C
Anticholinergics	Used in combination with beta agonists in infants with bronchospasm. Increased compliance and decreased respiratory system resistance [77]	II-3	C
Nutrition	Same as in Table 65.4A	I	B
Immunization	Prophylaxis against RSV and influenza decreases incidence of rehospitalization and morbidity	I	A

PMA post-menstrual age. From [56] with permission.

- Level I: Scientific evidence is lacking, of poor quality, or conflicting, such that the risk versus benefit balance cannot be assessed.

65.8.1 Novel Approaches/Therapies

Allopurinol In a randomized controlled trial (RCT) of 400 preterm infants, allopurinol given for 1 week did not impact on the incidence of BPD (at 28 days).

Alpha-1 Proteinase Inhibitor (α1P1) Systemic treatment with α1P1 did not reveal any statistically significant differences in the rates of BPD between the treated and placebo groups of preterm infants.

Continuous Tracheal Gas Insufflation (CTGI) In a prospective RCT in 34 premature infants, CTGI was not found to decrease death or BPD, compared to controls.

Cromolyn Two trials did not show any decrease in the incidence of BPD with the use of cromolyn (a mast cell stabilizer), which was confirmed in a meta-analysis.

Hydrocortisone Low–dose hydrocortisone did not improve survival without BPD [46]. The RCT had to be terminated early due to the hydrocortisone-treated infants receiving indomethacin having more gastrointestinal perforations [46].

Inositol In a meta-analysis of RCT using inositol supplementation in premature infants, the outcome of death or BPD was reported in two trials, and was found to be significantly reduced [47].

Liquid Ventilation In an uncontrolled study of 13 premature neonates treated with partial liquid ventilation, among 8 infants who survived till 36 weeks PMA, 4 had BPD.

Macrolide Antibiotics Erythromycin has not been shown to decrease BPD in infants when treated prophylactically or after known Ureaplasma colonization. Azithromycin, in a pilot study, did not reduce the incidence of BPD.

N-Acetyl Cysteine (NAC) In a large RCT, use of a 6-day course of NAC did not prevent BPD or death in infants with BW <1000 grams.

Nasal Continuous Positive Airways Pressure (NCPAP) Early surfactant replacement therapy with extubation to NCPAP compared with later selective surfactant replacement and continued mechanical ventilation with extubation from low ventilator support was associated with less need for mechanical ventilation, lower incidence of BPD and fewer air leak syndromes [48]. Use of a cut-off value of FiO2 of 0.45 to decide on transient intubation and surfactant replacement appeared to decrease the incidence of air leaks and BPD [48]. The Cochrane review, however, did not include the results of the COIN Trial, which reported that in infants born at 25–28 weeks' gestation, early nasal CPAP did not significantly

reduce the rate of death or BPD, as compared with intubation and the CPAP group had a higher incidence of pneumothoraces [49]. The NICHD-sponsored Neonatal Research Network SUPPORT Study, which looked at the same issue, in addition to optimal oxygen saturation ranges, did not find a difference in death or BPD.

Nasal Intermittent Positive Pressure Ventilation (NIPPV) NIPPV, both in the synchronized (SNIPPV) [50] and non-synchronized [51] modes, has been found to decrease BPD in RCT. The on-going NIP Trial is a large multicenter international RCT attempting to confirm the findings of the above-mentioned initial studies.

Nitric Oxide Inhaled nitric oxide (iNO) may or may not be beneficial in preventing BPD [52]. It does appear to be safe in this population, though it does not appear to impact on surfactant composition or pulmonary function. Routine use of iNO to decrease BPD in the preterm population is not recommended, pending long-term outcome data.

Patient-Triggered Ventilation Studies done to date have not shown any reduction in the incidence of BPD.

Recombinant CC10 Use of an anti-inflammatory protein, recombinant human Clara cell 10 kDa protein (CC10) has shown some initial promise.

Recombinant SOD Although the antioxidant recombinant Cu-Zn superoxide dismutase (SOD), did not show any difference in outcome, infants <27 weeks of gestation that received SOD had decreased hospitalizations, emergency room visits and less frequent use of bronchodilator therapy at age 1 year, as compared to infants that did not receive it [53].

Synthetic Surfactant A new synthetic surfactant (lucinactant), which contains the novel peptide, sinapultide, a surfactant-associated protein B mimic, appears to be similar to animal-derived surfactant in 2 multicenter randomized clinical trials. A meta-analysis of the studies revealed no statistically different differences in the outcomes of death and BPD [54].

Surfactant Delivery An attractive alternative to intubation and delivery of intra-tracheal surfactant would be aerosol delivery. To date, no effective aerosolized surfactant preparation is available for routine use in the clinical setting.

Thyroxine In a RCT, postnatal supplementation of thyroxine in preterm newborns did not decrease the incidence of BPD.

Vitamin E In a RCT, oral vitamin E supplementation did not reduce BPD in infants with BW <1500 grams.

Volume-Targeted Ventilation In a meta-analysis comparing volume-targeted ventilation to pressure-limited ventilation, there was no difference in the incidence of BPD, between the 2 groups.

More research is needed with these approaches before definite recommendations can be made.

65.9 Prevention

Among the antenatal factors, prevention of premature birth is the single most effective preventive measure for BPD. Understanding the biology of, and targeting therapies to inhibit, preterm labor safely would be an important goal. The use of progesterone in prevention of premature labor has shown promise, although the prolongation of gestation with this approach has yet to improve infant outcomes.

Antenatal steroids still remains the single most effective intervention for lung maturation and is recommended for clinical use. The impact of antenatal steroids on BPD has been somewhat controversial with studies reporting no or some benefit; other factors may perhaps mask the benefit of antenatal steroids. Weekly courses of antenatal steroids are not recommended. Betamethasone is preferred over dexamethasone.

Considerable research has gone into understanding the role of antenatal and postnatal inflammation in the pathogenesis of BPD [12, 13]. Antenatal interventions to treat chorioamnionitis have not decreased BPD; postnatal interventions may be too late to be effective. In most circumstances, maintenance of target oxygen saturation ranges >85–<95% is prudent in preterm neonates to avoid oxygen toxicity in an attempt to prevent the development of BPD, though achieving intended targets may be difficult. Restrictive fluid intake to meet physiological needs without resulting in significant dehydration showed a trend towards a lower risk for BPD [55]. Use of non-invasive methods of ventilation appears to show early promise in the prevention of BPD.

Given the complex nature of BPD (Fig. 65.1), it is unlikely that a single magic bullet (with the possible exception of prevention of preterm birth) will make a highly significant impact on BPD. Incremental improvements are possible by targeting multiple aspects of this condition include early diagnosis and treatment of antenatal and postnatal sepsis, optimal maintenance of oxygenation and fluid/electrolyte status, aggressive early parenteral/enteral nutrition, along with appropriate ventilatory strategies.

65.10 Conclusions

Focusing on aspects of basic biology (lung development, identification of biomarkers, genetics, animal models), and pharmacotherapeutic approaches [2] has the maximum potential to make an impact on BPD. Significant improvements in the future will likely depend on our ability to identify the genetic components of BPD and to target specific therapies.

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66

Rare Lung Diseases

Paolo Tagliabue and Clotilde Farina

66.1 Introduction

Rare lung diseases include a wide spectrum of conditions and are important causes of morbidity and mortality in newborn babies and infants. Despite the low incidence of these conditions, neonatologists are likely to encounter these disorders because they present with respiratory distress. For intensive care staff, radiologists, pathologists, and surgeons, the management and care of newborns affected by these diseases can require considerable time and resources within a tertiary care center. Embryological, pathological, surgical, antenatal and pediatric classifications for rare lung diseases have been reported [1–3]. The causes of rare respiratory diseases in neonates may be classified as: (a) Bronchial ultrastructural anomalies (e.g., primary ciliary dyskinesia [Kartagener's syndrome]), parenchymal molecular defects (e.g., congenital surfactant protein abnormalities), vascular malformations (e.g., congenital pulmonary lymphangiectasia and alveolar capillary dysplasia); (b) Developmental parenchymal abnormalities (e.g., pulmonary hypoplasia and cystic lung diseases [Table 66.1]).

The approach to rare lung diseases in neonates involves consideration of the prenatal history, clinical features and radiologic findings. A positive family history, ultrasonographic chest findings, polyhydramnios and associated congenital anomalies are suggestive. Clinical features may include: term gestation, unexpected respiratory distress, untreatable hypoxia without cyanotic heart defects, recurrent pulmonary infections or chronic lung disease of unknown origin. Relevant radiologic findings are chest asymmetry, dextrocardia, mediastinal shift, opacities or hyperlucencies, cystic pulmonary masses or fluid-filled lesions (Table 66.2).

Table 66.1 Rare pulmonary causes for respiratory distress in neonates

Parenchymal conditions
Primary ciliary dyskinesia (Kartagener's syndrome)
Congenital surfactant protein abnormalities
– Surfactant protein-B gene mutations
– Surfactant protein-C gene mutations
– ABCA3 transporter gene mutations
Congenital pulmonary lymphangiectasia
Alveolar capillary dysplasia
Developmental abnormalities
Lung agenesis
Pulmonary hypoplasia
Congenital diaphragmatic hernia
Cystic lung diseases
– Cystic adenomatoid malformation
– Bronchopulmonary sequestration
– Bronchogenic cyst
– Congenital lobar emphysema
Mechanical abnormalities
Rib cage anomalies
– Skeletal
– Muscular
– Central nervous
Chylothorax
Extrapleural and mediastinal masses

66.2 Parenchymal Diseases

66.2.1 Primary Ciliary Dyskinesia (PCD)

An autosomal recessive disease characterized by congenital impairment of mucociliary transport. This genetically, functionally, and ultrastructurally heterogeneous disease affects 1:20–30,000 individuals. Situs inversus is present in nearly 50% of cases. PCD rarely presents during the neonatal period and there is considerable variability in the severity of the clinical phenotype. Clinical manifestations include chronic respiratory infections beginning in early childhood. There is a

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Table 66.2 Approach to rare lung diseases in neonates

Prenatal history
Positive familiar history
Prenatal ultrasonographic chest findings
Polyhydramnios
Associated congenital anomalies
Neonatal clinical features
Term newborn
Unexpected respiratory distress
Untreatable hypoxia without cyanotic heart defects
Recurrent pulmonary infections
Chronic lung disease of unknown origin
Radiologic findings
Asymmetric chest imaging
Dextrocardia
Mediastinal shift
Radiological opacities
Radiological hypertransparencies
Cystic pulmonary masses
Fluid-filled lesions

history of neonatal respiratory distress in 43% of patients, and 55% have had recurrent lower respiratory tract infections from birth onwards.

The single most effective test is light microscopy of transnasal brushings for the ciliary beat pattern. Normal ciliary function excludes the diagnosis of PCD. Abnormality of the ciliary ultrastructure may be a primary defect or secondary to infections.

The mainstays of treatment are chest physiotherapy and the prompt and prolonged treatment of intercurrent respiratory infections, monitoring of hearing and oto-rhino-laryngological procedures.

The prognosis for a child with PCD is good. It is not considered to be a life-limiting condition and the outcome is very different from that of cystic fibrosis (CF) [4].

66.2.2 Congenital Surfactant Protein Abnormalities

Recessive mutations affecting surfactant protein-B (SP-B) and the ATP-binding cassette family member A3 (ABCA3) genes present as lethal surfactant deficiencies in the newborn, whereas other recessive mutations of ABCA3 and dominant mutations of the surfactant protein-C (SP-C) gene result in interstitial lung disease in older infants. Deficiency of surfactant protein B (SP-B) is the most common (Fig. 66.1). Shortly after birth, term infants continue to suffer from severe respiratory distress despite mechanical ventilation and repeated surfactant replacement. The only effective therapy is lung transplantation, without which the infants die within few months [5].

66.2.3 Congenital Pulmonary Lymphangiectasia (CPL)

A congenital malformation, with presentation from the fetus to early adulthood. CPL results from a failure of the pulmonary interstitial connective tissue to regress, leading to dilatation of lymphatic capillaries. Radiological findings include diffuse thickening of the peribronchovascular interstitium and the interlobular septa with pleural effusions. Supportive therapy includes albumin infusions, diuretics, thoracocentesis, and paracentesis. Nutrition plays an important role in reducing lymph production. Enteral nutrition with medium-chain triglycerides and total parenteral nutrition has been used successfully. CPL is often associated with congenital and genetic diseases, including Noonan, Ullrich-Turner, Ehlers-Danlos, and Down syndromes. In rare localized disease, surgical resection is curative. If presentation is during the newborn period, the clinical course is likely to be fatal.

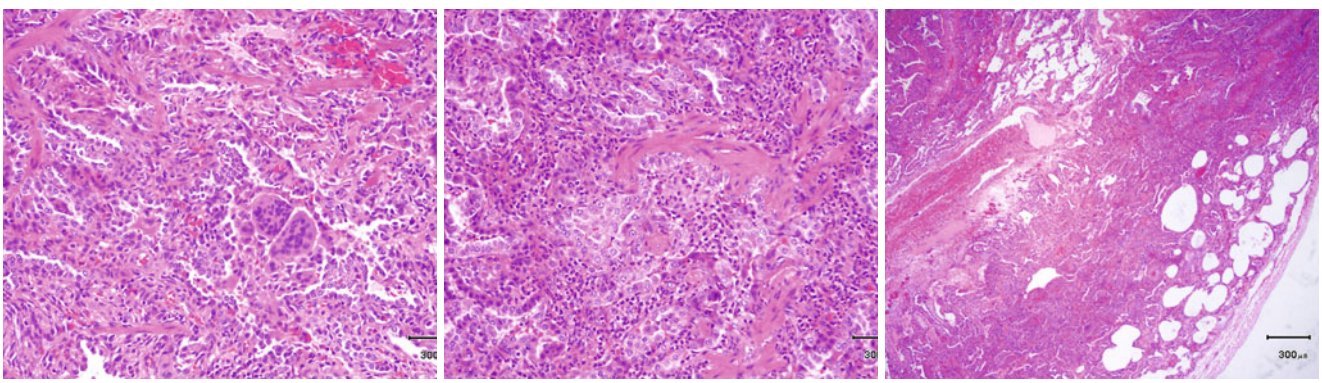

Fig. 66.1 Histopathology of a lung disorder caused by ABCA3 gene deletion. Alveolar proteinosis, admixed with alveolar macrophages, and thickened alveolar septa are seen in a biopsy from a neonate with fatal lung disease who was homozygous for a large ABCA3 gene deletion transmitted by consanguineous parents. (Personal case, by courtesy of Dr. V. Lucchini, pathologist)

For the majority of patients with neonatal presentations, gradual improvement and survival are possible, particularly if there are no significant co-existing abnormalities [5, 6].

66.2.4 Alveolar Capillary Dysplasia (ACD)

A disorder of pulmonary vascular development associated with persistent pulmonary hypertension in the newborn (PPHN) and unremitting hypoxemia that is unresponsive to pulmonary vasodilators and various modes of mechanical ventilation. Autosomal recessive inheritance is suspected. The diagnosis of ACD is confirmed at autopsy in 90% of cases, and in 10% by lung biopsy. Failure of development of alveolar capillaries leads to absence of a normal air-blood barrier. ACD is characterized by paucity of capillaries adjacent to the alveolar epithelium, anomalous distended veins, immature alveolar development and muscularization of the arterioles. Pathological features are diffuse in 85% and patchy in 15% of subjects. The symptoms and timing of presentation are related to the distribution of capillary dysplasia and the extent of alveolar underdevelopment. More than 95% are full-term with normal transition. Respiratory distress progressing to untreatable respiratory failure is the most common presentation. The onset of abnormal clinical signs is within the first hours of age in half the cases, while presentation at 2–6 weeks is reported in the 14% of cases. There is an association with other congenital malformations in 80% of cases, the commonest being gastrointestinal (30%), cardiac (30%), and renal anomalies (23%). If here is a high index of suspicion, diagnostic lung biopsy could be considered. ACD is generally fatal [7, 8].

66.2.5 Pulmonary Hypoplasia

In this condition, alveoli are reduced in number or size. Severe hypoplasia may be incompatible with extrauterine life. Lung growth before birth is dependent on blood supply, availability of space, respiratory movements, and fluid filling the airways in utero. Malformations of the rib cage, pleural effusions, thoracic masses or intestinal loops in congenital diaphragmatic hernia (CDH) compete with the developing lung for space. Adequate amniotic fluid is essential for normal lung development and conditions that produce oligohydramnios lead to diminished lung growth. Significant and prolonged oligohydramnios can result from chronic loss of liquor after preterm premature rupture of membranes, or from inadequate production or excretion of urine because of renal and urinary tract malformations: airway and arterial branching is inhibited, limiting the surface available for gas exchange. In the oligohydramnios sequence, the common phenotype is a flattened nose, contractures and growth impairment of extremities, known as Potter's syndrome.

Prolonged preterm premature rupture of membranes is not universally lethal but depends on the degree of pulmonary hypoplasia. The following conditions increase the risk of mortality:

- premature rupture of membranes at less than 25 weeks' gestation,
- severe oligohydramnios (amniotic fluid index <4) for more than 2 weeks,
- preterm delivery earlier than 28 weeks' gestation.

Serial amnioinfusions may be helpful if there is oligohydramnios. Abnormalities of the thoracic cage, with a small and bell shaped chest, are typical radiological findings. Lung hypoplasia is usually complicated by pulmonary hypertension and high-frequency ventilation and early use of inhaled nitric oxide therapy may be useful [1, 9].

66.3 Cystic Lung Diseases

Cystic lung lesions are the most common pulmonary lesions detected by routine antenatal ultrasound scanning. Different entities, such as congenital cystic adenomatoid malformation (CCAM), bronchopulmonary sequestrations (BPS), bronchogenic cyst (BC) and congenital lobar emphysema (CLE), may be difficult to differentiate, and antenatal MRI can provide more detail. A postnatal chest CT scan is useful in confirming the presence and extent of the lesions. Small lesions are usually asymptomatic both in utero and after birth. Large lesions may cause a mass effect resulting in esophageal compression and polyhydramnios, pulmonary hypoplasia, or vena caval obstruction with fetal hydrops. In these cases, fetal intervention may include thoraco-centesis or thoraco-amniotic shunting. The postnatal presentation is variable and depends on the size, location and type of lesion. Some infants may develop PPHN and respiratory failure. If there is associated pulmonary hypoplasia or ECMO is required, mortality is increased.

66.3.1 Congenital Cystic Adenomatoid Malformation (CCAM)

A congenital hamartomatous lesion of the lung, accounting for approximately 25% of congenital lung lesions. The incidence of CCAM is reported as between 1 in 11,000 and 1 in 35,000 live births. CCAM consists of a multicystic mass of dilated bronchiolar-like spaces that proliferate at the expense of alveoli. The cysts that enlarge following air and fluid trapping cause compression of the adjacent normal lung and mediastinum.

CCAM is categorized into four types. Type 1 is characterized by a few large cysts and is the most common (75%). In type 2, there are evenly spaced small cysts. Type 3 is very rare and appears solid on gross examination. Type 4

is characterized by acinar-type epithelium rather than the bronchiolar epithelium. Usually only one lobe is involved.

Enlarging lesions may cause progressive signs because of expansion of the cysts shortly after birth. Alternatively, abnormal signs may develop later if there are intercurrent infections. The neonatal presentation therefore ranges from respiratory failure (affecting approximately 50% of cases) to a healthy asymptomatic infant.

Large lesions may compromise the normal development of the fetal lung, resulting in pulmonary hypoplasia or neonatal death. CCAM is a cause of fetal hydrops and polyhydramnios. Infants with highly symptomatic CCAMs require surgery. Most commonly, this occurs in neonates presenting with respiratory failure of sufficient severity to require ventilatory support. When considering the timing of surgery for asymptomatic infants, some clinicians favor operating during the neonatal period but others prefer to wait until the child is between 6 months and 2 years of age. The prognosis depends on the size of the lesion, the degree of development of the adjacent lung, and the presence of other congenital anomalies [3, 5, 10, 11].

66.3.2 Bronchopulmonary Sequestration (BPS)

Composed of abnormal lung tissue without connection to the normal tracheo-bronchial tree. There are two types of BPS based on their relationship to the pleurae: extra- and intralobar. Both receive an anomalous arterial supply from the systemic circulation, usually a branch of the aorta.

With extralobar BPS, the mass of pulmonary parenchyma is outside the pleurae. The lesion is found between the left lower lobe and the diaphragm in 66% of the cases. More common in males (3:1), 50% of newborns have respiratory distress because of compression of the rest of the lung parenchyma. In more than 65%, there are associated anomalies, including CDH (30%), pericardial defects, and anomalous pulmonary venous return.

Intralobar BPS is characterized by a lesion within the lobe of the lung without separate pleurae. Intralobar BPS accounts for 75% of all BPS. Usually in the lower lobe (95%), on antenatal US, BPS appears as a solid mass. The presence of hydrops or polyhydramnios are poor prognostic factors. The majority of BPS (68%) undergo spontaneous regression before birth and survival is 95%. Early complications are usually related to the degree of associated pulmonary hypoplasia, but also result from other associated anomalies. Large BPS or BPS with high systemic blood flow are indications for resection. Lobectomy is the treatment of choice for intralobar BPS [2 ,3, 8].

66.3.3 Bronchogenic Cyst (BC)

Commonly mediastinal, it is a solitary cyst filled with fluid or mucus, attached and not communicating with the tracheobronchial tree. Lined with pseudostratified ciliated columnar epithelium with goblet cells and enlarged with mucous, these are considered to be infected peripheral lesions. Treatment is by lobectomy or excision of the cyst only [3].

66.3.4 Congenital Lobar Emphysema (CLE)

Caused by an intrinsic or extrinsic obstruction of a lobar bronchus with progressive air trapping and overinflation of the affected areas. Histologically, the lung parenchyma is normal with enlargement of the airspaces. Usually diagnosed postnatally by 6 months of age. The upper lobes are involved in 90% of cases and it is more common in males (2:1). In 40% of CLE, there are associated cardiovascular anomalies. If there is respiratory distress, the affected part of the lung should be removed. Spontaneous resolution has been reported and asymptomatic cases may be followed expectantly. The prognosis is generally good and depends on whether there are associated anomalies [3, 5].

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67

Persistent Pulmonary Hypertension of the Newborn and Congenital Diaphragmatic Hernia

Steven H. Abman

67.1 Introduction

Persistent pulmonary hypertension of the newborn (PPHN) is a clinical syndrome that represents the failure to achieve or sustain the normal decrease in pulmonary vascular resistance (PVR) at birth, leading to severe respiratory distress and hypoxemia. PPHN is a major clinical problem, affecting nearly 10% of full-term infants admitted to neonatal intensive care units, and contributes significantly to high morbidity and mortality [1, 2]. Newborns with PPHN are at risk for severe asphyxia and its complications, including death, chronic lung disease, neurodevelopmental sequelae, and other problems. This chapter will review the pathophysiology and treatment of pulmonary hypertension in the setting of PPHN, including newborns with congenital diaphragmatic hernia (CDH).

67.2 Insights into PPHN from the Laboratory

Diverse animal models have been used in order to better understand the pathogenesis and pathophysiology of PPHN. Such models have included exposure to acute or chronic hypoxia after birth, chronic hypoxia in utero, placement of meconium into the airways of neonatal animals, sepsis and others. Each model demonstrates interesting physiologic changes that may be especially relevant to particular clinical settings, but most studies only examine brief changes in the pulmonary circulation, and mechanisms underlying altered lung vascular structure and function of PPHN remain poorly understood. Neonates with severe PPHN who die during the first days after birth already have pathologic signs of chronic pulmonary vascular disease, suggesting that intrauterine events may play an important role in this syndrome [3]. Adverse intrauterine stimuli during late gestation, such as abnormal hemodynamics, changes in substrate or hormone delivery to the lung, hypoxia, inflammation or others, may potentially alter lung vascular function and structure, contributing to abnormalities of postnatal adaptation. Several investigators have examined the effects of chronic intrauterine stresses, such as hypoxia or hypertension, in animal models in order to attempt to mimic the clinical problem of PPHN. Whether chronic hypoxia alone can cause PPHN is controversial. There has been a report that maternal hypoxia in rats increases pulmonary vascular smooth muscle thickening in newborns, but this observation has not been reproduced in more extensive studies of maternal rats or guinea pigs [4]. Acute hypoxia alone is insufficient to account for PPHN, which is further reflected in recent clinical observations that PPHN is rare in patients with severe asphyxia who were enrolled in hypothermia studies.

Pulmonary hypertension induced by early closure of the ductus arteriosus (DA) in fetal lambs alters lung vascular reactivity and structure, causing the failure of postnatal adaptation at delivery, and provides an experimental model of PPHN [5, 6]. Over days, pulmonary artery pressure and PVR progressively increase, but flow remains low and PaO_2 is unchanged [6]. Marked right ventricular hypertrophy and structural remodeling of small pulmonary arteries develops after 8 days of hypertension. After delivery, these lambs have persistent elevation of PVR despite mechanical ventilation with high oxygen concentrations. Studies with this model show that chronic hypertension without high flow can alter fetal lung vascular structure and function. This model is characterized by endothelial cell dysfunction and abnormal smooth muscle cell vasoreactivity and growth, including findings of impaired NO production and activity and down-regulation of lung endothelial NO synthase mRNA and protein expression [7–9]. Fetal pulmonary hypertension is also associated with decreased cGMP concentrations, associated with decreased soluble guanylate cyclase and upregulated cGMP specific phosphodiesterase (PDE5) activities, suggesting further impairments in downstream signaling [10, 11]. Thus, multiple

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

alterations in the NO-cGMP cascade appear to play an essential role in the pathogenesis and pathophysiology of experimental PPHN, contributing to altered structure and function of the developing lung circulation, and leading to failure of postnatal cardio-respiratory adaptation. Recent evidence indicates that excessive production of reactive oxygen species (ROS), such as superoxide in the pulmonary vasculature may further contribute to the disruption in NO-cGMP signaling in this model, and may contribute to poor responsiveness to inhaled NO therapy [9, 12, 13].

Upregulation of ET-1 may also contribute to the pathophysiology of PPHN. Circulating levels of ET-1, a potent vasoconstrictor and co-mitogen for vascular smooth muscle cell hyperplasia, are increased in human newborns with severe PPHN [14]. In the experimental model of PPHN due to compression of the DA in fetal sheep, lung ET-1 mRNA and protein content is markedly increased, and the balance of ET receptors are altered, favoring vasoconstriction [15, 16]. Chronic inhibition of the ET-A receptor attenuates the severity of pulmonary hypertension, decreases pulmonary artery wall thickening, and improves the fall in PVR at birth in this model [17]. Thus, experimental studies have shown the important role of the NO-cGMP cascade and the ET-1 system in the regulation of vascular tone and reactivity of the fetal and transitional pulmonary circulation.

Oxidant stress plays an important role in the pathogenesis of PPHN. Increased ROS such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) have been demonstrated in pulmonary arteries in the ovine ductal ligation model of persistent pulmonary hypertension [12, 18]. Mitochondrial dysfunction, increased expression and activity of NADPH oxidase, and uncoupled eNOS activity can each generate ROS [12, 19–21]. Increased ROS production promotes vasoconstriction directly and through multiple mechanisms that may include increased endothelin levels [22] and oxidization of free fatty acids to create vasoconstrictor metabolites, such as isoprostanes [23]. O_2^- rapidly combines and inactivates NO and forms peroxynitrite, a potent oxidant with the potential to produce vasoconstriction and cytotoxicity. Increased ROS in the pulmonary vasculature of the ductal ligation model promotes dysfunction of NO-cGMP signaling at multiple steps in the pathway, including blunted eNOS expression, uncoupled eNOS activity (thus further promoting ROS production), and increased activity and expression of cGMP-specific phosphodiesterases, and impaired sGC activity [9, 13, 24]. Superoxide dismutases (SOD) catalyze the conversion of superoxide anions to H_2O_2 and O_2. Due to the efficiency of the reaction between NO and superoxide, the local concentration of SOD is a key determinant of the biological half-life of endogenous NO [25]. Evidence for a critical pathologic role for ROS in PPHN includes the recent observation that administration of a single intratracheal dose of rhSOD in neonatal lambs with PPHN produced a sustained increase in oxygenation over a 24 hour period, reduced production of isoprostanes and peroxynitrite, and restored normal eNOS expression and function.

In addition to vasoactive mediators, alterations of growth factors, such as VEGF and platelet-derived growth factor (PDGF), likely play key roles in PPHN. VEGF is markedly decreased in experimental PPHN, and treatment with recombiant human VEGF restores endothelial function and lowers PVR in this model [26–28]. In addition, inhibition of PDGF-B attenuates smooth muscle hyperplasia in experimental pulmonary hypertension in fetal lambs, suggesting a potential role in the pathogenesis of PPHN [26]. Additional new data suggest that maternal exposure to selective serotonin reuptake inhibitors (SSRI) during late gestation is associated with a six-fold increase in the prevalence of PPHN [29], although it is not clear how many infants developed severe disease. Newborn rats exposed in utero to fluoxetine develop pulmonary vascular remodeling, abnormal oxygenation and higher mortality when compared with vehicle-treated controls [30]. However, these findings showed only mild changes in right ventricular hypertrophy and pulmonary vascular remodeling in the neonatal rat pups, with minimal changes in vasoreactivity after maternal SSRI exposure. Whether these effects were related to direct impact on the fetal lung circulation or secondary to altered maternal or umbilical-placental physiology remains unknown. As SSRIs have been reported to reduce pulmonary vascular remodeling in adult models of pulmonary hypertension, these findings also serve to highlight the unique vulnerability of fetal pulmonary vascular development.

67.3 Clinical PPHN

The first reports of PPHN described term newborns with profound hypoxemia who lacked radiographic evidence of parenchymal lung disease and echocardiographic evidence of structural cardiac disease. In these patients, hypoxemia was caused by marked elevations of PVR leading to right–to-left extrapulmonary shunting of blood across the patent ductus arteriosus (PDA) or foramen ovale (PFO) during the early postnatal period. Due to the persistence of high PVR and blood flow through these fetal shunts, the term "persistent fetal circulation" was originally used to describe this group of patients. Consequently, it was recognized that this physiologic pattern can complicate the clinical course of neonates with diverse causes of hypoxemic respiratory failure. As a result, the term PPHN has been considered as a syndrome, and is currently applied more broadly to include neonates that have a similar physiology in association with different cardiopulmonary disorders, such as meconium aspiration, sepsis, pneumonia, asphyxia, congenital diaphragmatic hernia, respiratory distress syndrome (RDS), and others (Table 67.1).

Striking differences exist between these conditions, and mechanisms that contribute to high PVR can vary between these diseases. However, these disorders are included in the syndrome of PPHN due to common pathophysiologic features,

Table 67.1 Disorders associated with neonatal pulmonary hypertension

1. *Pulmonary*
   - Meconium aspiration syndrome
   - Respiratory distress syndrome
   - Lung hypoplasia
   - Congenital diaphragmatic hernia
   - Pneumonia/Sepsis
   - Idiopathic
   - Transient tachypnea of the newborn
   - Alveolar-capillary dysplasia
   - Associated abnormalities in lung development
   - – congenital lobar emphysema (rare association)
   - – cystic adenomatoid malformation (rare association)
   - – idiopathic, with impaired distal alveolarization
   - – pulmonary interstitial glycogenosis
   - – surfactant protein B and C deficiency
   - – ABCA 3 deficiency
   - – others
2. *Cardiovascular*
   - Myocardial dysfunction (asphyxia; infection; stress)
   - Anatomic cardiac diseases
   - Hepatic and cerebral arteriovenous malformations (AVMs)
   - Total anomalous pulmonary venous return
   - Pulmonary vein stenosis (isolated)
   - Pulmonary atresia
3. *Associations with Other Diseases*
   - Neuromuscular disease
   - Metabolic disease
   - Maternal drugs or smoking
   - – cyclooxygenase inhibitors
   - – selective serotonin reuptake inhibitors

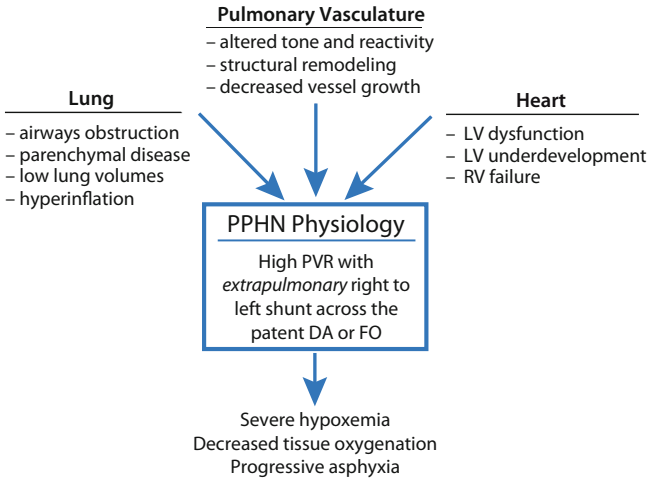

Fig. 67.1 Heart, lung and pulmonary vascular interactions in PPHN

including sustained elevation of PVR leading to hypoxemia due to right-to-left extrapulmonary shunting of blood flow across the ductus arteriosus (PDA) or foramen ovale (PFO). In many clinical settings, hypoxemic respiratory failure in term newborns is often presumed to be associated with PPHN-type physiology; however, hypoxemic term newborns can lack echocardiographic findings of extrapulmonary shunting across the PDA or PFO. Thus, PPHN should be reserved to describe neonates in whom extrapulmonary shunting contributes to hypoxemia and impaired cardiopulmonary function. Recent estimates suggest an incidence for PPHN of 1.9/1000 live births, or an estimated 7400 cases/year [31] (Fig. 67.1).

Diseases associated with PPHN are often classified into one of 3 categories: i) maladaptation: vessels are presumably of normal structural but have abnormal vasoreactivity; ii) excessive muscularization: increased smooth muscle cell thickness and increased distal extension of muscle to vessels which are usually non-muscular; and iii) underdevelopment: lung hypoplasia associated with decreased pulmonary artery number. This designation is imprecise, however, and high PVR in most patients likely involves overlapping changes among these categories. For example, neonates with congenital diaphragmatic hernia (CDH) are primarily classified as having vascular underdevelopment due to lung hypoplasia, yet lung histology of fatal cases typically shows marked muscularization of pulmonary arteries, and clinically, these patients can respond to vasodilator therapy. Similarly, neonates with meconium aspiration often have clinical evidence of altered vasoreactivity, but excessive muscularization is often found at autopsy.

As described above, autopsy studies of fatal PPHN demonstrate severe hypertensive structural remodeling even in newborns who die shortly after birth, suggesting that many cases of severe disease are associated with chronic intrauterine stress. However, the exact intrauterine events that alter pulmonary vascular reactivity and structure are poorly understood. Epidemiologic studies have demonstrated strong associations between PPHN and maternal smoking and ingestion of cold remedies that include aspirin or other non-steroidal anti-inflammatory products [32, 33]. Since these agents can induce partial constriction of the ductus arteriosus, it is possible that pulmonary hypertension due to antenatal ductal narrowing contributes to PPHN. Other perinatal stresses, including placenta previa and abruption, and asymmetric growth restriction, are associated with PPHN; however, most neonates who are exposed to these prenatal stresses do not develop PPHN. Circulating levels of L-arginine, the substrate for NO, are decreased in some newborns with PPHN, suggesting that impaired NO production may contribute to the pathophysiology of PPHN. It is possible that genetic factors increase susceptibility for pulmonary hypertension. A recent study reported strong links between PPHN and polymorphisms of the carbamoyl phosphate synthase gene [34]. However, the importance of this finding is uncertain and further work is needed in this area. Studies of adults with idiopathic primary pulmonary hypertension have identified abnormalities of bone morphogenetic protein (BMP) receptor genes; whether polymorphisms of genes for the BMP or TGF-β receptors, other critical growth factors, vasoactive substances

or other products increase the risk for some newborns to develop PPHN is unknown.

67.4 Clinical Physiology and Evaluation

Clinically, PPHN is most often recognized in term or near-term neonates, but clearly can occur in premature neonates as well. PPHN typically presents as respiratory distress and cyanosis within 6–12 hours of birth. While PPHN is often associated with perinatal distress, such as asphyxia, low APGAR scores, meconium staining, and other factors, idiopathic PPHN can present without signs of acute perinatal distress. Radiographic findings are variable, depending upon the primary disease associated with PPHN. Classically, the chest X-ray in idiopathic PPHN is oligemic, normally or slightly hyperinflated, and lacks parenchymal infiltrates. In general, the degree of hypoxemia is disproportionate to the severity of radiographic evidence of lung disease.

Not all term newborns with hypoxemic respiratory failure have PPHN physiology [35]. Hypoxemia in the newborn can be due to extrapulmonary shunt, as described above, in which high pulmonary artery pressure at systemic levels leads to right-to-left shunting of blood flow across the PDA or PFO. However in many infants intrapulmonary shunt or ventilation-perfusion mismatch, is the predominant abnormality, in which hypoxemia results from the lack of mixing of blood with aerated lung regions due to parenchymal lung disease, without the shunting of blood flow across the PDA and PFO. In the latter setting, hypoxemia is related to the amount of pulmonary arterial blood that perfuses non-aerated lung regions. Although PVR is often elevated in hypoxemic newborns without PPHN, high PVR does not contribute significantly to hypoxemia in these cases.

Several factors can contribute to high pulmonary artery pressure in neonates with PPHN-type physiology. Pulmonary hypertension can be due to vasoconstriction or structural vascular lesions that directly increase PVR. Changes in lung volume in neonates with parenchymal lung disease can also be an important determinant of PVR. PVR increases at low lung volumes due to dense parenchymal infiltrate and poor lung recruitment, or with high lung volumes due to hyperinflation associated with overdistension or gas–trapping. Cardiac disease is also associated with PPHN. High pulmonary venous pressure due to left ventricular dysfunction (e.g., asphyxia or sepsis) can also elevate PAP, causing right-to-left shunting, with little vasoconstriction. In this setting, enhancing cardiac performance and systemic hemodynamics may lower PAP more effectively than promoting pulmonary vasodilation. Thus, understanding the cardiopulmonary interactions is key to improving outcome in PPHN.

PPHN is characterized by hypoxemia that is poorly responsive to supplemental oxygen. In the presence of right-to-left shunting across the PDA, "differential cyanosis" is often present, which is difficult to detect by physical exam, and is defined by a difference in PaO_2 between right radial artery versus descending aorta values >10 torr, or an O_2 saturation gradient >5%. However, post-ductal desaturation can be found in ductus-dependent cardiac diseases, including hypoplastic left heart syndrome, coarctation of the aorta or interrupted aortic arch. The response to supplemental oxygen can help to distinguish PPHN from primary lung or cardiac disease. Although supplemental oxygen traditionally increases PaO_2 more readily in lung disease than cyanotic heart disease or PPHN, this may not be obvious with more advanced parenchymal lung disease. Marked improvement in SaO_2 (increase to 100%) with supplemental oxygen suggests the presence of V/Q mismatch due to lung disease or highly reactive PPHN. Most patients with PPHN have at least a transient improvement in oxygenation in response to interventions such as high levels of inspired oxygen and/or mechanical ventilation. Acute respiratory alkalosis induced by hyperventilation to achieve $PaCO_2$ <30 torr and a pH >7.50 may increase PaO_2 >50 torr in PPHN, but rarely in cyanotic heart disease.

The echocardiogram plays an important diagnostic role and is a useful tool for managing newborns with PPHN. The initial echocardiographic evaluation rules out structural heart disease causing hypoxemia or ductal shunting (e.g., coarctation of the aorta and total anomalous pulmonary venous return). Further, as stated above, not all term newborns with hypoxemia have PPHN physiology. Although high pulmonary artery pressure is commonly found in association with neonatal lung disease, the diagnosis of PPHN is uncertain without evidence of bidirectional or predominantly right-to-left shunting across the PFO or PDA. Echocardiographic signs suggestive of pulmonary hypertension (e.g., increased right ventricular systolic time intervals and septal flattening) are less helpful. In addition to demonstrating the presence of PPHN physiology, the echocardiogram is critical for the evaluation of left ventricular function and diagnosis of anatomic heart disease, including such PPHN mimics as coarctation of the aorta; total anomalous pulmonary venous return; hypoplastic left heart syndrome; and others. Studies should carefully assess the predominant direction of shunting at the PFO as well as the PDA. Although right-to-left shunting at the PDA and PFO is typical for PPHN, predominant right-to-left shunting at the PDA but left-to-right shunt at the PFO may help to identify the important role of left ventricular dysfunction to the underlying pathophysiology. In the presence of severe left ventricular dysfunction with pulmonary hypertension, pulmonary vasodilation alone may be ineffective in improving oxygenation. In this setting, efforts to reduce PVR should be accompanied by targeted therapies to increase cardiac performance and decrease left ventricular afterload. In the setting of impaired LV performance, cardiotonic therapies that increase systemic vascular resistance may further worsen LV function and increase pulmonary artery pressure. Thus, careful echocardiographic assessment provides invaluable information about the underlying pathophysiology and will help guide the course of treatment.

67.5 Treatment of PPHN

In general, therapy includes optimization of systemic hemodynamics with volume and cardiotonic therapy (dobutamine, dopamine, and milrinone), in order to enhance cardiac output and systemic O_2 transport. Failure to respond to medical management, as evidenced by the failure to sustain improvement in oxygenation with good hemodynamic function, often leads to treatment with extracorporeal membrane oxygenation (ECMO) [36]. Although ECMO can be a life-saving therapy, it is costly, labor intensive, and can have severe side effects, such as intracranial hemorrhage. Since arterio-venous ECMO usually involves ligation of the carotid artery, the potential for acute and long-term CNS injuries continues to be a major concern.

The goal of mechanical ventilation is to improve oxygenation, achieve optimal lung volume to minimize the adverse effects of high or low lung volumes on PVR, and to minimize the risk for lung injury (volutrauma). Mechanical ventilation using inappropriate settings can produce acute lung injury (ventilator-induced lung injury; VILI), causing pulmonary edema, decreased lung compliance and promotes lung inflammation due to increased cytokine production and lung neutrophil accumulation. The development of VILI is an important determinant of clinical course and eventual outcome of newborns with hypoxemic respiratory failure, and postnatal lung injury worsens the degree of pulmonary hypertension [37]. On the other hand, failure to achieve adequate lung volumes (functional residual capacity) contributes to hypoxemia and high PVR in newborns with PPHN. Some newborns with parenchymal lung disease with PPHN physiology improve oxygenation and decrease right-to-left extrapulmonary shunting with aggressive lung recruitment during high frequency oscillatory ventilation [38] or with an open lung approach of higher positive end-expiratory pressure with low tidal volumes, as more commonly utilized in older patients with ARDS [39].

Past studies have shown that acute hyperventilation can improve PaO_2 in neonates with PPHN, providing a diagnostic test and potential therapeutic strategy. However, there are many issues with the use of hypocarbic alkalosis for prolonged therapy. Depending upon the ventilator strategy and underlying lung disease, hyperventilation is likely to increase VILI, and the ability to sustain decreased PVR during prolonged hyperventilation is unproven. Experimental studies suggest that the response to alkalosis is transient, and that alkalosis may paradoxically worsen pulmonary vascular tone, reactivity and permeability edema [40, 41]. In addition, prolonged hyperventilation reduces cerebral blood flow and oxygen delivery to the brain, potentially worsening neurodevelopmental outcome. Overall, hyperventilation is currently not recommended for prolonged treatment of PPHN.

Additional therapies, including infusions of sodium bicarbonate, surfactant therapy and the use of intravenous vasodilators, are also highly variable between centers. Although surfactant improved oxygenation and reduced ECMO in some lung diseases, such as meconium aspiration and RDS, a multicenter trial showed benefit in infants with relatively mild disease, and failed to show a reduction in ECMO utilization in the subset of newborns with idiopathic PPHN [42]. The use of intravenous vasodilator drug therapy, with agents such as tolazoline, magnesium sulfate, prostacyclin and sodium nitroprusside, is also controversial due to the non-selective effects of these agents on the systemic circulation. Systemic hypotension may worsen right-to-left shunting, impair oxygen delivery and worsen gas exchange in patients with parenchymal lung disease. In addition, the initial response to agents such as tolazoline is often transient, and severe adverse effects such as gastrointestinal hemorrhage have been reported. Endotracheal administration of vasodilators, including tolazoline, sodium nitroprusside, and prostacyclin may cause selective pulmonary vasodilation and minimize systemic hypotension. However, these data are largely limited to animal studies, and evidence is needed to confirm the safety and efficacy of this approach in humans.

Inhaled nitric oxide (iNO) therapy at low doses (5–20 ppm) improves oxygenation and decreases the need for ECMO therapy in patients with diverse causes of PPHN (Fig. 67.2) [43–48]. Multicenter clinical trials support the use of iNO in near-term (>34 weeks gestation) and term newborns, although the use of iNO in infants less than 34 weeks gestation remains largely investigational. Studies support the use of iNO in infants who have hypoxemic respiratory failure with evidence of PPHN, who require mechanical ventilation and high inspired oxygen concentrations. The most common criterion employed has been the oxygenation index (OI; mean airway pressure times FiO_2 times 100 divided by PaO_2). Although clinical trials commonly allowed for enrollment with OI levels >25, the mean OI at study entry for these studies approximated 40. Whether treatment at lower OI levels reduces ECMO use is uncertain [49] (see Chapter 71).

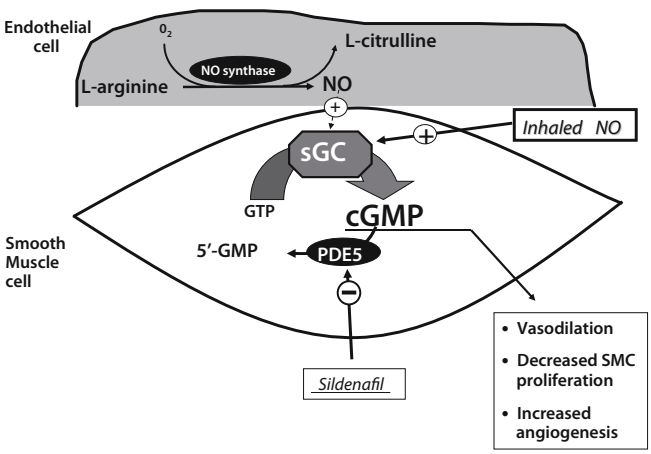

Fig. 67.2 Mechanisms of NO-cGMP Therapy in PPHN

In newborns with severe lung disease, HFOV is frequently used to optimize lung inflation and minimize lung injury. The combination of HFOV with iNO often enhances the improvement in oxygenation in newborns with severe PPHN complicated by diffuse parencyhmal lung disease and under inflation (e.g., RDS, pneumonia). A randomized, multicenter trial of infants with severe PPHN demonstrated that treatment with iNO in combination with HFOV was successful in many patients who failed to respond to HFOV or iNO alone [46]. For patients with PPHN complicated by severe lung disease, response rates for HFOV+iNO were better than HFOV alone or iNO with conventional ventilation. In contrast, for patients without significant parenchymal lung disease, both iNO and HFOV+iNO were more effective than HFOV alone. This response to combined treatment with HFOV+iNO likely reflects both improvement in intrapulmonary shunting in patients with severe lung disease and PPHN (using a strategy designed to recruit and sustain lung volume, rather than to hyperventilate) and augmented NO delivery to its site of action. Although iNO may be an effective treatment for PPHN, it should be considered only as part of an overall clinical strategy that simultaneously addresses the role of parenchymal lung disease, cardiac performance, and systemic hemodynamics.

Although clinical improvement during inhaled NO therapy occurs with many disorders associated with PPHN, not all neonates with acute hypoxemic respiratory failure and pulmonary hypertension respond to iNO. Several mechanisms may explain the clinical variability in responsiveness to iNO therapy. As noted above, an inability to deliver NO to the pulmonary circulation due to poor lung inflation is a major cause of poor responsiveness. In addition, poor NO responsiveness may be related to myocardial dysfunction or systemic hypotension, severe pulmonary vascular structural disease, and unsuspected or missed anatomic cardiovascular lesions (such as total anomalous pulmonary venous return, coarctation of the aorta, alveolar capillary dysplasia, pulmonary interstitial glycogenosis, surfactant protein deficiency, and others). Since iNO is usually delivered with high concentrations of oxygen, there is also the potential for enhanced production of reactive oxygen and reactive nitrogen metabolites, both of which may contribute to vasoconstriction and/or inadequate responses to iNO.

While hyperoxic ventilation is standard therapy for PPHN, high FiO_2 may be toxic to the developing lung through formation of ROS [47]. As noted above, iNO responsiveness may be blunted after even brief (30 minutes) periods of ventilation with 100% O_2, and that oxidant stress alters NO responsiveness in part through increasing expression and activity of cGMP-specific phosphodiesterases [24, 50].

The pulmonary vasodilator response to iNO is mediated primarily by the generation of cGMP after activation of soluble guanylate cyclase (sGC). This effect is limited by high PDE5 activity, which rapidly metabolizes cGMP, and decreases the ability of iNO or other agonists to sustain vasodilation [51]. Recently approved by the FDA for the treatment of adult chronic pulmonary hypertension, sildenafil is a potent and highly specific PDE5 inhibitor that has been used in several preclinical studies in animal models. For instance, in lambs with experimental pulmonary hypertension, both enteric and aerosolized sildenafil dilate the pulmonary vasculature and augment the pulmonary vascular response to iNO [52, 53]. Intravenous sildenafil was found to be a selective pulmonary vasodilator with efficacy equivalent to inhaled nitric oxide in a piglet model of meconium aspiration, although hypotension and worsening oxygenation resulted when sildenafil was used in combination with iNO [54, 55]. Data have only recently begun to emerge on the use of sildenafil alone or in combination with iNO in clinical populations, and investigations in newborn infants have been limited because sildenafil is currently available only in enteric form. A recent report demonstrated that enteric sildenafil improved oxygenation and survival in human infants with PPHN compared to placebo [56]. Preliminary findings from a pilot study of intravenous sildenafil in newborns with pulmonary hypertension indicate that the infusion was generally well tolerated, with improvements in oxygenation noted in the cohorts that received higher infusion doses [57].

Prostacyclin is a potent vasodilator that causes pulmonary vasodilation through stimulation of adenylate cyclase to increase intracellular cAMP levels. Although iv prostacyclin may worsen gas exchange in PPHN patients with lung disease and systemic vasodilation, inhaled PGI_2 may enhance oxygenation in infants that are poorly responsive to iNO [58]. Another potential approach taking advantage of cAMP signaling is inhibition of phosphodiesterase type 3 (PDE3) that metabolizes cAMP. Milrinone, a PDE3 inhibitor, has been shown to decrease pulmonary artery pressure and resistance, and to act additively with iNO in animal studies. A recent report indicates that the addition of intravenous milrinone to neonates with severe PPHN and poor iNO responsiveness was associated with improvements in oxygenation without compromising hemodynamic status [59]. Milrinone is of additional benefit by lowering systemic afterload in the setting of PPHN newborns with poor LV function.

67.6 Pulmonary Vascular Disease in Congenital Diaphragmatic Hernia

Advances in our understanding of PPHN physiology and its treatment has led to marked reductions in morbidities and the need for ECMO therapy. However, ECMO remains an effective and potentially life-saving rescue modality for severe PPHN. Of multiple diseases associated with PPHN, neonates with congenital diaphragmatic hernia (CDH) are often refractory to therapy and continue to have high ECMO use and mortality. CDH occurs in roughly 1:3,000 births and is a complex disease of varying degrees of severity, which includes elements of lung hypoplasia with reduced alveolarization and

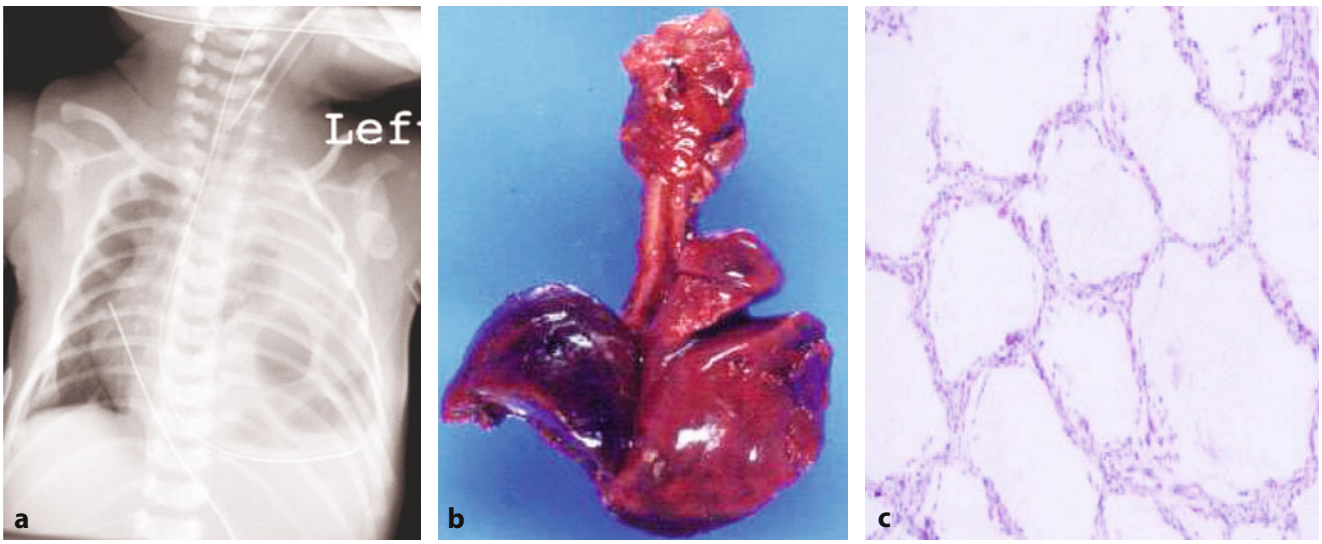

Fig. 67.3 Congenital diaphragmatic hernia (CDH): radiographic (**a**), anatomic (**b**) and histologic (**c**) aspects

lung size; pulmonary vascular disease; and cardiac dysfunction (Fig. 67.3) (reviewed in [60]).

Pulmonary vascular disease in CDH is more severe than most forms of PPHN due to the combinations of high tone and abnormal vasoreactivity, structural remodeling of the vessel wall and marked reduction in vascular density associated with lung hypoplasia [61]. Data from experimental models and clinical studies have clearly shown that abnormalities of lung vascular growth during late fetal life play a significant role in the pathophysiology of CDH and long-term outcomes. For example, fetal echocardiography and MRI studies have shown that decreased left pulmonary artery blood flow and impaired vasodilation with brief maternal hyperoxia in utero is strongly associated with the severity of lung hypoplasia and mortality [62]. In addition, lung histology of infants who die early with CDH and pulmonary hypertension shows substantial vascular remodeling (Fig. 67.4). Severe pulmonary hypertension in CDH is not due to reduced vascular growth alone, and intrauterine stimuli lead to marked hypertensive structural changes as well.

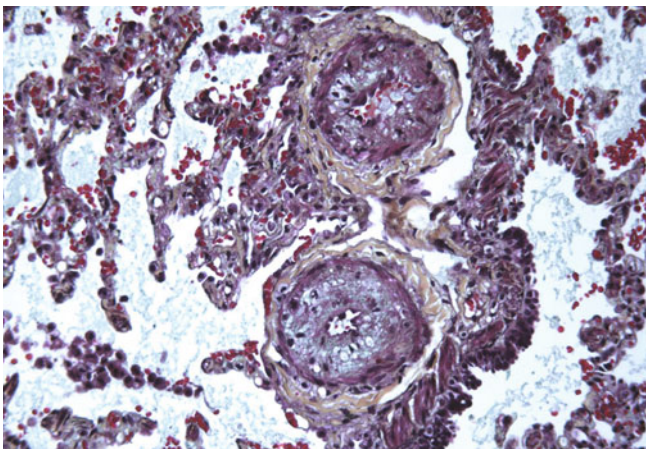

Fig. 67.4 Lung hystology of infant with CDH and PPHN

Early descriptions of inhaled NO in CDH infants showed that some infants have marked and sustained increases in PaO_2 with reduction of PVR, suggesting that high tone contributed to pulmonary hypertension in these cases. However, clinical trials suggest poor responsiveness in CDH overall [63]. Differences in outcomes regarding inhaled NO therapy between babies and centers suggest variability in disease severity, reflects different components of cardiac, pulmonary and vascular components to their disease (as discussed above; Fig. 67.1), differences in management between centers and other factors. Perhaps a major factor is the relative contribution of more severe structural lung vascular disease in non-responders. Such structural features, including smooth muscle cell hyperplasia, adventitial thickening and intimal occlusion (in severe cases), along with reduced lung alveolar-vascular surface area, contribute to the failure of neonates with severe CDH to respond to conventional therapies as well as patients with other causes of PPHN [43]. In addition to difficulties with pulmonary hypertension during the early neonatal period, infants with CDH are also at risk for prolonged and late pulmonary hypertension (Table 67.2) [61].

Sustained pulmonary hypertension in CDH, despite aggressive therapies, is strongly linked with high mortality [64]. Recent approaches to improve outcomes with severe CDH include earlier use of HFOV, delayed repair until evidence of hemodynamic stability and improved pulmonary hypertension is achieved, the use of prostaglandin E1 to maintain ductal patency with severe disease, augmentation of LV function with milrinone, and the use of additional pulmonary hypertension therapies beyond inhaled NO alone. These latter

Table 67.2 Stages of pulmonary hypertension in congenital diaphragmatic hernia

– Early (hours to weeks) - severe PPHN, but with variable contributions of lung disease and LV abnormalities (size, function)
– Late (weeks to months) - PH remains high, often near systemic levels, which can persist despite weaning from mechanical ventilation
– Chronic (months to years) - significant PH despite stable respiratory course

agents include sildenafil and bosentan, which are being used more readily in severe or late pulmonary hypertension in CDH infants [65]. More studies are needed to improve pulmonary hypertension therapies to enhance short and long-term outcomes of infants with CDH.

67.7 Summary

PPHN is a clinical syndrome that is associated with diverse cardiopulmonary diseases, with pathophysiologic mechanisms including pulmonary vascular, cardiac and lung disease. Experimental work on basic mechanisms of vascular regulation of the developing lung circulation and models of perinatal pulmonary hypertension has improved our therapeutic approaches to neonates with PPHN. Inhaled NO has been shown to be an effective pulmonary vasodilator for infants with PPHN, but successful clinical strategies require meticulous care of associated lung and cardiac disease. More work is needed to expand our therapeutic repertoire in order to further improve the outcome of the sick newborn with severe hypoxemia, especially in patients with CDH and advanced structural vascular disease.

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68

Treatment of Respiratory Failure: Mechanical Ventilation

Colin J. Morley

68.1 Introduction

Respiratory diseases, with different etiologies, are one of the major problems in neonatal medicine and one of the main reasons for an infant receiving intensive care support. Without respiratory support many ill newly born infants would die.

Respiratory support can be given with several different techniques. From the least intensive to the most intensive they are: additional oxygen, continuous positive airways pressure (CPAP), bi-level CPAP or nasal ventilation, untriggered mechanical ventilation, triggered mechanical ventilation and high frequency oscillatory ventilation. This chapter will only present information about mechanical ventilation.

In any discussion of mechanical ventilation it is important to define the terminology of spontaneous inspiration and mechanical ventilation because this can be confusing.

It is important to realize that only people have breaths and ventilators deliver inflations.

- A *breath* is a spontaneous inspiration by an infant.
- An *inspiration* is a breath taken by the baby.
- An *inflation* is a pressure applied to the airways by a ventilator with the intention of inflating the lungs.
- *Spontaneous expiration* is gas leaving the lung after a breath.
- *Ventilator expiration* is gas leaving the lung after an inflation.
- *Inspiratory time* is the time during which a baby inspires.
- *Inflation time* is the duration of a mechanical inflation.
- *Ventilator expiration time* is the expiration time set on the ventilator.
- *Expiration time* is the time gas leaves the lungs after an inspiration or expiration until a new inflation or inspiration occurs.

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68.2 Problems with Mechanical Ventilation of Infants

Mechanical ventilation is associated with a number of problems so it is most important not to ventilate an infant unless it is absolutely necessary.

The problems with ventilation are:

- Gas is pushed into the lungs rather than sucked in. This is unphysiological. It is much more effective for lung aeration and blood circulation through the lungs to have a negative pressure with each inspiration.
- The endotracheal tube (ETT) by passes the larynx. This may lower the residual lung volume because the larynx is very important for maintaining lung volume during lung disease. During expiration it may narrow or close to reduce the expiratory flow and maintain lung volume [1].
- An ETT increases resistance to gas flow and breathing.
- Inflations can injure the airways and alveoli [2]. This is by several mechanisms. 1) Volutrauma where the tidal volume is too high and the over-distension injures the lungs [3]. 2) Repeated expansion and collapse of atelectatic areas of the lung – called atelectrauma [4]. 3) Barotrauma where repeated high ventilator pressures damage the lungs. 4) Injury from the ETT in the airway.
- Ventilation increases infection.
- Mechanical ventilation may interfere with the baby's control of breathing and blood gases.
- We still do not have good rules about when to start ventilation, how to prevent lung damage, the optimal way to wean the ventilation and extubate the infant, or when to stop ventilation.

68.3 Indications for Mechanical Ventilation

The major reason for ventilating an infant is apnea. However, the difficult decision comes with deciding when to ventilate an infant who is very premature, or breathing with retraction

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of the lower chest wall, or requiring oxygen treatment, or having numerous apneic episodes.

Intubation and ventilation of very premature babies with respiratory difficulty soon after birth used to be considered mandatory. However, it is now realized that even very premature babies can often be supported with CPAP alone particularly if treated early with caffeine [5].

68.3.1 Apnea

Apnea unresponsive to stimulation, nasal CPAP, caffeine and hand ventilation for a short time, is an absolute indication for mechanical ventilation.

There are few other absolute guidelines about when to ventilate a baby. It is often a matter of clinical judgment and local policy, considering the infant's gestational age, and other factors.

68.3.2 Respiratory Failure

The first question is does the infant have respiratory failure? An arterial $PaCO_2$ that has risen above 60 mmHg (~8 kPa) is a good sign of respiratory failure. However, this judgment should not be made on the first blood gas measurement, unless the baby is obviously very ill, because many babies improve over the first hour or two with careful and gentle management and after the baby has been appropriately positioned and nasal CPAP has been used.

A capillary CO_2 is not accurate enough for making the decision to ventilate an infant because it might be spuriously high in an infant with poor peripheral circulation.

A severe uncompensated metabolic acidosis may be an indication for ventilation.

68.3.3 Oxygenation

A high and rising FiO_2, despite treatment with nasal CPAP, is a strong pointer to the need for ventilation. There is little consensus about the FiO_2 that should be used to determine when a baby should be ventilated. To a certain extent the FiO_2 does not matter if the PaO_2 is maintained. However, a high FiO_2 is a sign that there is a low lung volume. Oxygen is toxic to the lungs and the higher it is the more likely lung injury occurs. Pragmatically, an FiO_2 of 0.60 or higher is an indication for ventilation in a very premature infant treated with CPAP [6].

The criteria for ventilation may vary depending on the size of the baby. Babies born at <26 weeks gestation may need earlier ventilator support because of their extreme immaturity. More mature babies, are stronger and ventilation should not be used unless absolutely necessary.

68.4 Investigations

68.4.1 Arterial Blood Gas Measurements

Understanding and assessing arterial blood gases is one of the most important aspects of the care of a baby with respiratory failure. Unless the baby has mild RDS, as indicated by a low FiO_2 and minimal dyspnoea, an arterial line should be inserted to enable easy and repeated measurement of arterial blood gases. A ventilated baby will require several accurate blood gases in the first days and so an arterial line is optimal for the baby and carers. Capillary blood gases can be misleading and venous blood gases should not be used to assess the respiratory function.

68.4.2 Chest X-ray

An anterior/posterior chest X-ray should be done as early as possible to confirm the respiratory diagnosis and exclude diagnoses that may need different treatment. If umbilical arterial and/or venous lines have been used the X-ray should include the abdomen to show their position.

68.4.3 Investigate Infection

Infection must always be suspected in babies presenting with respiratory failure soon after birth because it cannot be excluded, even in babies born by cesarean section. Infection at this age usually causes septicemia and can be quickly life threatening if antibiotics are not given quickly. Blood cultures, gastric aspirate microscopy and culture, and blood tests for indicators of infection should be taken and then intravenous antibiotics given. Penicillin and gentamycin are safe and effective.

68.5 The Basic Principles of Mechanical Ventilation

Several aspects of ventilation need to be adjusted to optimize the blood gases, lung volume, tidal volume and minute volume, and minimize lung injury.

68.5.1 Improving Oxygenation

Oxygenation can be improved by increasing the FiO_2. However, the higher the FiO_2 the greater the chance of lung injury because high levels are toxic to the epithelium. Therefore

other techniques should be used to improve oxygenation and reduce the need for a high FiO_2.

Oxygenation is not dependant on the movement of gas in and out of the lungs. It only requires oxygen to enter the lungs and an appropriate surface area for diffusion.

Adequate oxygenation depends on ensuring the lungs are expanded with a good surface area for gas exchange [7]. Oxygenation is closely related to the PEEP or mean airway pressure used because this is the key factor to maintain lung volume in an intubated infant with lung disease. The level of PEEP should be between 5 and 10 cmH_2O. There is no easy guide to the exact level to use in any baby at any time. PEEP can be increased when a high FiO_2 is used or when the chest X-ray shows low lung volume.

Controlling the PEEP depends on clinical assessment, chest X-ray appearance and FiO_2. PEEP can be increased to open the lungs if the FiO_2 is high and the chest X-ray also shows the lungs to be under inflated. The levels of PEEP to use in different situations have not been determined.

Altering peak pressure or inflation and expiration times will alter the mean airway pressure, and have some effect on oxygenation, but adjusting these is not as effective at altering oxygenation as changing PEEP.

In a normal lung high PEEP may cause oxygenation to deteriorate by compressing the alveolar capillaries. However, there is no evidence this is a problem in babies ventilated for respiratory failure unless increased to a very high level.

Oxygenation is dependant on the lungs being appropriately perfused. Inadequate oxygenation may be due to low blood pressure or pulmonary hypertension. Therefore to ensure optimal pulmonary perfusion and oxygenation the blood pressure must be maintained.

Primary pulmonary hypertension, with right to left shunting of venous blood past the lungs, can be recognized when a baby has a high FiO_2 to obtain a satisfactory PaO_2 yet the $PaCO_2$ is easily controlled. The chest X-ray shows lungs with a normal volume and reduced vascular markings. Echocardiography is used to confirm the diagnosis.

One problem to be aware of is when the peak inflating pressure (PIP) is progressively increased to try and improve oxygenation but the oxygenation deteriorates. This may be because the pressure is causing over-distension and interfering with pulmonary blood flow. A pointer to this is that oxygenation improves when the baby is transiently disconnected from the ventilator.

68.5.2 Controlling the Blood Level of Carbon Dioxide

CO_2 control is related to moving gas in and out of the lung. Inadequate spontaneous breathing is the primary reason for ventilation. Altering the tidal volume and ventilator rate controls the $PaCO_2$. Careful control of the $PaCO_2$ is very important. Over ventilation causing hypocarbia ($PaCO_2$ <30 mmHg) is strongly associated with chronic lung disease and adverse neurodevelopmental outcomes [8]. Hypercarbia ($PaCO_2$ >60 mmHg) may also be damaging because it causes acidosis and increased cerebral perfusion. The normal $PaCO_2$ range for a ventilated baby is about 35–60 mmHg.

During ventilation the tidal volume is determined by the applied pressure i.e., PIP – PEEP and the baby's breathing. Neonatologists think of neonatal ventilation in terms of applied pressures and the effect they have on the $PaCO_2$, however the pressure is a proxy for the tidal volume. The higher the PIP the greater the tidal volume delivered. Most modern neonatal ventilators measure and display tidal volume so this can now be targeted. Large tidal volumes can damage the lungs very quickly – called volutrauma. The appropriate tidal volume for ventilated spontaneously breathing babies is around 3.5–6 mL/kg depending on the ventilator rate. If the $PaCO_2$ is too high or too low the tidal volume (or peak inflating pressure) is altered a little (0.5 mL/kg or 2 cmH_2O) and the $PaCO_2$ rechecked in about 30 minutes.

The ventilator rate should be adequate to ensure the $PaCO_2$ is in the normal range. Most babies are ventilated while breathing. They breathe between 50 and 90/min. Modern ventilators enable inflations to be triggered and synchronized with each breath to assist the baby's respiratory efforts. This is called assist control mode of ventilation. This means that the baby triggers inflations and controls the ventilator rate and to some extent the minute volume. With triggered ventilation the back-up rate should be set at about 30/min, well below the baby's spontaneous rate. The product of ventilator rate and tidal volume is the minute volume. The minute volume should be ~ 200–300 mL/kg/min.

The $PaCO_2$ is related to the minute volume and so it can be increased or decreased by altering ventilator rate. However, because ventilated babies are spontaneously breathing and triggering the ventilator, altering the set rate may have little effect on the delivered minute volume.

68.5.3 Heating and Humidification of Ventilation Gases

Inspired gases are heated and humidified by the nose, pharynx and upper respiratory mucosa. They are delivered to the lungs at 37°C and with a saturation of 100%.

Medical gases are 100% dry and cold. An ETT by-passes the upper airway and so all heating and humidification of the gases must be from a heater humidifier in the circuit. Even slightly cold gases or dry gases quickly thicken the mucus and damage the mucosa. It is important to ensure that all gas reaches the lungs at no less than 37°C and carries 44 mg% H_2O (100% relative humidity) [9]. Any condensation of water vapor from the inspired gas is a loss of humidity and must be avoided by using a heated inspiratory ventilator circuit.

68.5.4 Measuring Blood Gases

During mechanical ventilation, adjusting the ventilator settings in response to frequently measured blood gases is necessary for control of $PaCO_2$ and pH. The frequency depends on the stability of ventilation. In the acute phase, when the lung function is changing, blood gases should be measured no more than 30 minutes after a change in ventilator settings. Transcutaneous measurements of CO_2 can be useful, give quick feed back about changes in CO_2 and reduce the number of blood gases measured but are prone to drift.

68.5.5 Monitoring Ventilator Parameters

Most modern ventilators have numerical and graphical displays of ventilator parameters. It is important these are understood and interpreted correctly. The most useful graphical display is pressure, flow and tidal volume against time with a horizontal axis showing about ten seconds worth of waveforms. See Figs. 68.1–68.6 for recordings during different ventilator modes and their interpretation [10].

68.5.5.1 Pressure Wave

This shows the ventilator pressure wave changing with inflations. It shows the level of PEEP and how this changes with spontaneous inspirations, the onset of inflation, the rate of rise of the pressure, whether the set PIP is achieved and whether there is any pressure plateau.

68.5.5.2 Flow Wave

This shows the gas flow changing as the baby breathes and the ventilator inflates. Flow above the zero line is down the ETT, flow below the zero line is flow out of the ETT. It displays: a small inspiratory flow immediately before inflation that triggers inflation; any flow from spontaneous breaths not associated with inflations; the gas flow during inflations; enables comparison of inflation and expiratory flow curves and whether there is any gas leak during inflation; any interaction between the spontaneous breaths and inflations; whether the inflation time is adequate; whether the expiratory time is appropriate for full expiration; whether there is gas trapping; diaphragmatic braking; or any obstruction or splinting against the inflations.

68.5.5.3 Tidal Volume Wave

This shows the volume of gas passing down the ETT as an up stroke, the volume of gas leaving the lung as a downward curve and any gas leak around the ETT as a vertical line at the end of each inflation.

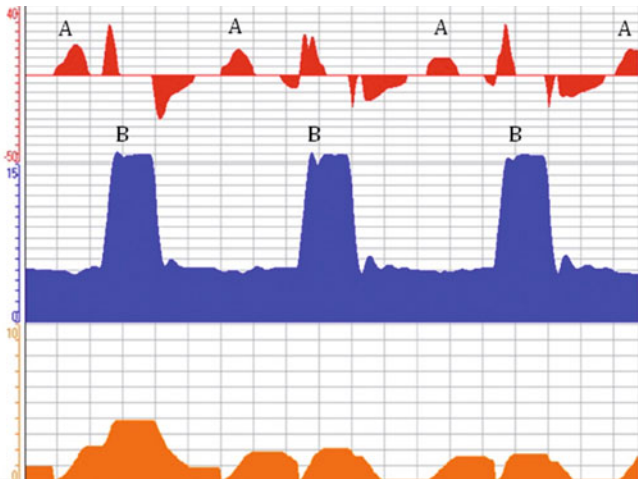

Fig. 68.1 This shows about 3.8 seconds of a recording of CMV with the flow wave at the top with gas going down the ETT above the zero line and gas coming out of the ETT below the zero line. The pressure wave is in the middle showing 5 cmH_2O PEEP pressure and 16 cmH_2O peak pressure. The bottom line shows the tidal volume wave. At "A" the baby is inspiring, the PEEP falls slightly and there is an inspiratory tidal volume. At "B" the ventilator pressure rises to a plateau for the inflation time of about 0.3 sec. This results is an inflationary tidal volume about the same size as the baby's inspiratory volume. It can be seen that ventilator inflations and baby's inspirations and expirations are completely out of synchrony.

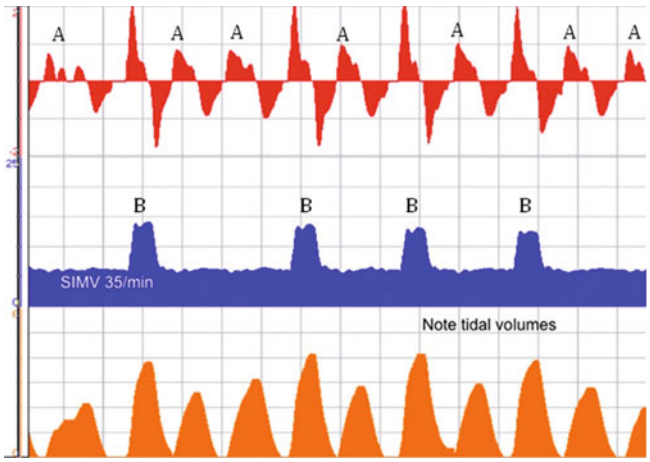

Fig. 68.2 This shows about 9 seconds of a recording of SIMV at a rate of 35/minute with the flow wave at the top with gas going down the ETT above the zero line and gas coming out of the ETT below the zero line. The pressure wave is in the middle showing about 6 cmH_2O PEEP pressure and 14 cmH_2O peak pressure. The bottom line shows the tidal volume wave. At "A" the baby is inspiring without ventilator inflations. There is an inspiratory and expiratory tidal volume of about 2–3 mL. At "B" the ventilator pressure rises to a plateau for the inflation time of about 0.3 sec. This results in an inflationary tidal volume about 20% larger than the baby's tidal volume. What cannot be seen is that these inflations were triggered by the baby's inspiratory flow and so the tidal volumes are a combination of the baby's breathing and synchronous inflation. The baby is breathing at about 75 breaths per minute. It can be seen that baby makes about seven inspirations without ventilator support and four with ventilator support. Out of a combined tidal volume during this time of about 34 mL the ventilator has contributed only 4 mL. It would appear that this baby probably does not need to be ventilated.

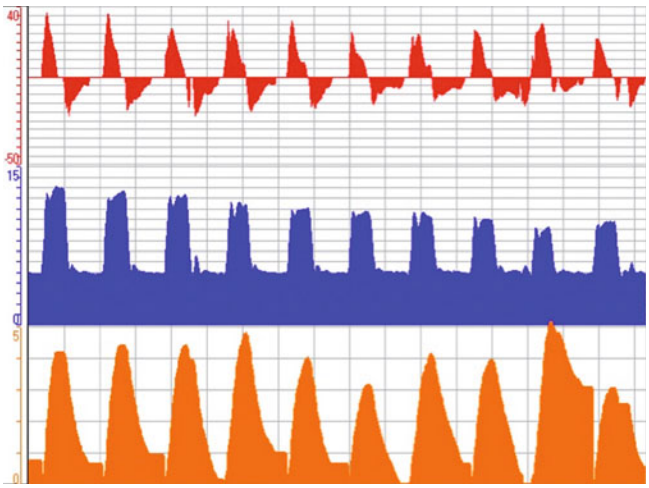

Fig. 68.3 This shows about 9 seconds of a recording of A/C (SIPPV) and volume guarantee mode at a set rate of 50 /min with the flow wave at the top with gas going down the ETT above the zero line and gas coming out of the ETT below the zero line. The pressure wave is in the middle showing about 6 cmH_2O PEEP pressure and the PIP changing from 13 down to 11 cmH_2O. The bottom line shows the tidal volume wave. Each of the baby's inspirations triggered an inflation and drove the ventilator at about 65/min. There is an inspiratory and expiratory tidal volume varying slightly between 4 mL and 6 mL with variable a leak. This shows how well A/C ventilation supports each of the baby's breaths and how volume guarantee changes the pressure to try and maintain the tidal volume as the baby changes its breathing.

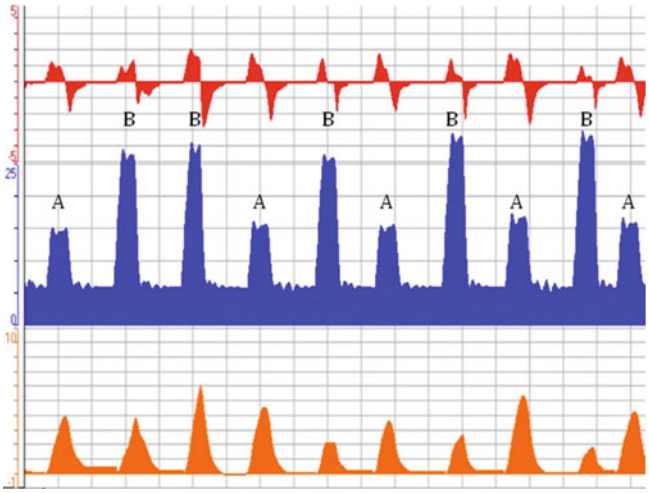

Fig. 68.4 This shows about 9 seconds of a recording of A/C (SIPPV) and volume guarantee mode at a set rate of 50/min with the flow wave at the top with gas going down the ETT above the zero line and gas coming out of the ETT below the zero line. The pressure wave is in the middle showing about 6 cmH_2O PEEP pressure. The PIP changes from about 15 cmH_2O to about 30 cmH_2O. The bottom line shows the tidal volume wave. The waves marked A are triggered inflations with a lower PIP and the waves marked B are untriggered inflations with a much higher PIP. This is happening because the volume guarantee program targets triggered and untriggered PIP separately. The pressures are changing slightly to try and produce the set tidal volume of 4.5 mL. Note even though there is a large difference in the driving pressure the tidal volume for triggered and untriggered inflations are similar and sometimes the untriggered inflations with the higher PIP have the smaller tidal volumes. There is an inspiratory and expiratory tidal volume varying slightly between 4 mL and 6 mL with variable a leak. This shows the problem of having the back up rate very close to the baby's spontaneous rate. The ventilator frequently changes from triggered to untriggered inflations.

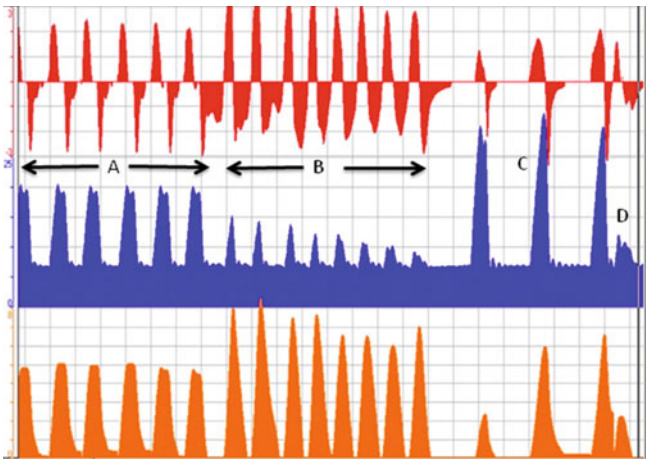

Fig. 68.5 This shows about 13 seconds of a recording of A/C (SIPPV) and volume guarantee mode at a set rate of about 50/min with the flow wave at the top with gas going down the ETT above the zero line and gas coming out of the ETT below the zero line. The pressure wave is in the middle showing about 7 cmH_2O PEEP pressure. The PIP changes to try and maintain the set tidal volume 5 mL. The bottom line shows the tidal volume wave. The group of waves marked A are all triggered inflations with a stable tidal volume. The group of waves marked B are all triggered inflations but here the tidal volume has suddenly increased because the baby started breathing hard with inflation tidal volume of more than 130% of the set tidal volume. The volume guarantee program reacts by stopping the inflations. Note their inflation times are shorter. The PIP is lower and reducing each inflation to try and bring the tidal volumes back to the set level. At C there are 3 large inflations at a slower rate. These are untriggered inflations at the back-up rate and have PIPs that correspond to the PIP of previous untriggered inflations. At D the baby inspires and a much smaller triggered PIP is delivered. This all shows the dynamic nature of the volume guarantee program responding to large changes in the infants breathing pattern.

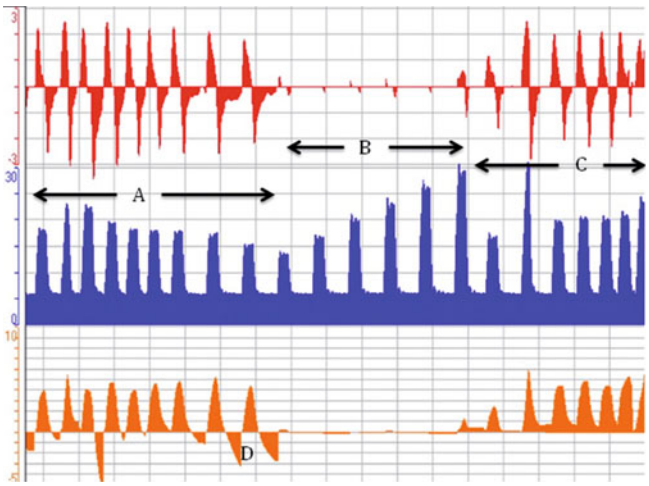

Fig. 68.6 This figure shows A/C volume guarantee ventilation. At A the inflations are all triggered and the PIP changes a little to maintain the tidal volume. At B the infant splints against the ventilator inflations and stops the flow and tidal volumes even though an inflation is being delivered. As the tidal volume is lower than set the volume guarantee program uses untriggered, back-up, inflations and increases the PIP by 3 cmH_2O every inflation until the baby starts breathing again at C, except for one untriggered inflation. Note at D there are expiratory tidal volumes larger than the inspiratory ones. This often happens just as the baby starts splinting and blowing down the lung volume.

68.6 Techniques of Mechanical Ventilation

This section will be illustrated from my experience and studies with using the Drager Babylog 8000+ ventilator and the Sensormedics 3100A high frequency oscillating ventilator for many years [11–26]. Other neonatal ventilators may have slightly different modes but the general principles of use are the same.

68.6.1 Conventional Mandatory Ventilation (CMV) or Intermittent Mandatory Ventilation (IMV)

CMV and IMV are exactly the same mode and use pressure limited, time cycled ventilation. The ventilator is adjusted to deliver a set PIP and a set PEEP with every inflation. There is no single PIP or PEEP that can be used for all babies under all circumstances. The starting PIP is chosen by experience e.g., 20 cmH_2O and the infant's chest wall movement assessed by observation. If this is considered to be too much the PIP is reduced. If there is none, or too little chest wall movement the PIP is increased. When changing PIP by observing chest movement remember in normal babies the movement with each breath is barely discernable. Subsequently, the PIP is adjusted according to the $PaCO_2$. There is no easy way to determine the appropriate PEEP for a baby and this usually starts at 5 cmH_2O.

The inflation time is usually set to 0.3 sec, as this is the mean inspiratory time of a ventilated preterm infant. If it is set longer the infant may breathe out during the ventilator inflation. The expiratory time is set to provide the desired respiratory rate, ~ 60 per minute.

See Fig. 68.1 for a recording of ventilator, flow and tidal volume waves during CMV showing the asynchrony between inflations and inspirations.

This is the only ventilator mode available on transport ventilators.

There are many problems with this mode of ventilation.

- It is unresponsive to the infant's respiratory efforts because it is not triggered or synchronized with the infant's breathing. It may inflate when the infant is breathing out or it may inflate on top of a large inspiration resulting in a very large tidal volume.
- The set PIP may be too high or too low for the infant resulting in over or under ventilation. Over ventilation can cause high tidal volumes, acute lung injury and the resulting hypocarbia is associated with periventricular leukomalacia.
- It provides little or no monitoring of the actual ventilation the baby receives.
- The ventilator rate cannot match the infant's respiratory rate, particularly as it changes.

68.6.2 Triggered Ventilation

The purpose of triggered ventilation is for the ventilator to inflate the infant in synchrony with the infant's inspiration and augment the inspiratory gas flow and tidal volume. With the Drager Babylog 8000 + ventilator triggering is from a hot wire flow sensor which is placed between the Wye piece of the ventilator circuit and the ETT. Two fine tungsten wires heated to 400°C detect the gas flow by the cooling effect of the gas. It detects inspiratory gas flow and when this has reached about 0.2 L/min, ~ 30 ms after the beginning of inspiratory flow an inflation is started. The delay time depends on the set trigger sensitivity on a scale of 1 to 10 with 1 being the most sensitive. This sensitivity should always be set to 1 so that the inflation occurs as close as possible to the onset of the baby's inspiration. A higher number will mean a longer delay between the onset of inspiration and the start of inflation resulting in a non-synchronous inflation.

A potential problem with flow triggering is that the sensor detects gas flow from condensed water bubbling in the ventilator circuit and this triggers an inflation even though the baby may not be inspiring at this time – so called auto-triggering. Some people reduce the sensitivity to prevent this, however, this causes an unacceptable delay between the onset of inspiration and the inflation. Ensuring the ventilator circuit is free of condensed water can prevent this.

Two other trigger mechanisms have been used.

1. Uses a capsule stuck on the abdomen to detect abdominal movement. This is unreliable. The effectiveness and accuracy depends on where it is placed on the abdomen. It may not detect the start of an inspiration. On many occasions it triggers on expiration.
2. Using a change in the pressure in the ventilator circuit depends on the baby making a sufficiently large inspiration to change the circuit pressure (~0.5 cmH_2O). It is inaccurate if the baby is very small or has a low inspiratory effort.

Some people worry that adding a flow sensor increases the dead space and thereby increases the infant's $PaCO_2$. This is rarely a problem because as an uncuffed ETT is sued there is almost always a leak around the ETT. Even with no leak the effect on $PaCO_2$ is small and not of clinical significance compared with the gains from using the sensor.

68.6.3 Synchronised Intermittent Ventilation (SIMV)

In this mode the ventilator is a pressure limited, time cycled ventilator but inflations are triggered by the baby's inspiratory gas flow in synchrony with a set number of inspirations. This means that not all the baby's inspirations are supported by a ventilator inflation e.g., if the baby is breathing at 80/min and the ventilator is set to deliver 30/min only 30 of the breaths will coincide with an inflation. For all other breaths the inspiration is through the ETT with only PEEP for support. It the baby is apneic the set ventilator rate is delivered.

The ventilator divides each minute by the set number of inflations, called the ventilator interval, e.g., if set to 60/min because the inflation time is 0.3 s and the expiratory time 0.7 s then the ventilator interval is 1 sec. If the rate is set to 30/min, inflation time 0.3 s and expiratory time 1.7 s the ventilator interval is 2 sec. The ventilator will deliver each inflation, triggered or untriggered, within each set ventilator interval.

See Fig. 68.2 for a recording of the pressure, flow and tidal volume waves during SIMV.

The problems with SIMV ventilation are:

- Not all inspirations are supported by an inflation.
- The unsupported breaths through the ETT increase the work of breathing and can lead to exhaustion.
- The tidal volume delivered may vary considerably from the triggered inflations to the spontaneous breaths and also due to the set PIP being delivered on top of the baby's tidal volumes.

When should SIMV be used? Because of the problems with spontaneous breaths not being supported by the ventilator it is inappropriate to use SIMV when the baby is initially ventilated or in respiratory failure. It may have a place when weaning ventilation but this can be a problem if the ventilator rate is set so low that the majority of the baby's breaths are unsupported. It has not been shown to be superior to A/C (SIPPV).

68.6.4 Assist Control (A/C) also Called Synchronised Intermittent Positive Pressure Ventilation (SIPPV)

In this mode inflations are triggered by all the baby's inspirations. So it inflates the baby in synchrony with each spontaneous inspiration. This is the most appropriate mode to use when the baby requires full ventilatory support [27–29].

It is pressure limited, time cycled ventilation and the PIP, PEEP, inflation time and expiratory time are set. However if the rate is less than the baby's rate the baby's inspiration controls the rate.

It is important to realize the purpose of A/C is to support all inspirations. If the ventilator rate (the back-up), is set too high the baby does not have enough time to make a breath before the mandatory inflation starts, the spontaneous breaths are overridden by the ventilator and the ability to trigger inflations and assist the infant is lost, e.g., if the back-up rate is 60/min then the infant will only trigger inflations if it breathes faster than 60/min. At this back-up rate only about half the baby's breaths will trigger an inflation [22]. It is "confusing" for the baby to be trying to breathe and suddenly have an inflation asynchronous with its inspirations. The main purpose of the A/C ventilation is lost. Therefore it is important to set the back-up rate slower, about 30/min, so all the baby's breaths can trigger an inflation. If the baby stops breathing the ventilator rate will be 30/min. Spontaneously breathing ventilated babies rarely become completely apneic for very long but obviously a rate of 30/min for several minutes after a spontaneous triggered rate of about 70/min would need careful monitoring.

See Fig. 68.3 for a recording of the pressure, flow and tidal volume waves during A/C ventilation.

People have told to me they worry that with A/C mode babies are more likely to become over ventilated if they trigger the ventilator all the time. In my experience this is no more of a problem than with other modes if the PIP is set to ensure reasonable average tidal and minute volumes. The clinicians need to carefully monitor the tidal volume and minute volume.

In A/C mode the baby mainly controls the ventilator rate and so if the $PaCO_2$ is low it is inappropriate to reduce the rate to reduce minute volume because the baby is controlling the rate. To control $PaCO_2$ PIP is the parameter to change.

68.6.5 Volume Guarantee Ventilation

One of the major complications from ventilating infants is acute lung injury leading to bronchopulmonary dysplasia. Volutrauma is one of the main causes of this. Tidal volumes of more than 8 mL/kg can cause volutrauma and so it is important to measure and control the tidal volume. This mode reduces volutrauma.

Traditionally, during neonatal ventilation altering the PIP has been used to change the $PaCO_2$. It is often forgotten, or not realized, that the PIP is a proxy for tidal volume and altering the PIP changes the tidal volume. The delivered PIP can now be changed to target a specified tidal volume. This should be in the range of 3.5–6.0 mL/kg depending on the baby's breathing rate and the ventilator back-up rate.

The volume guarantee mode specifically targets the delivery of an expiratory tidal volume set by the clinicians [30]. It works in all triggered modes: SIMV, A/C and PSV. The ventilator monitors expired tidal volume and determines whether it is higher, lower or identical to the set expired tidal volume. If it is higher the PIP is reduced for the next inflation and if the tidal volume is lower the PIP is increased to try and ensure the set tidal volume is achieved.

The volume guarantee mode controls the expired tidal volume very closely to the set tidal volume [30]. Analyses from 6693 inflations: mean (SD) for triggered expired tidal volume = 102% (29%) of set, range 0–378%; for untriggered = 97% (31%), range 0–322% [22]. It can be seen that there can be large variations in the tidal volume. This is due to the infant's breathing. If the infant takes breaths larger than the set tidal volume the ventilator cannot, and should not, prevent this. The ventilator response is to lower the PIP for subsequent inflations. If the inspired tidal volume is more than 130% then the inflation is stopped. Sometimes ventilated infants "splint" or contract their abdominal muscles very hard and completely stop an inflation. The response of the ventilator will be to increase the PIP in a stepwise fashion to try and restore the tidal volume. This overcomes the splinting, and consequent hypoxia, quicker than ventilation continuing with a set PIP.

See Figs. 68.3, 68.4, 68.5 and 68.6 for recordings of assist control with volume guarantee.

Some pressure limited ventilators have two inflating pressures called pressure support ventilation. In this mode the PIPs are set and not related to the delivered tidal volume for many inflations. However, in volume guarantee mode the PIP changes all the time to try and maintain the tidal volume in response to the infant's breathing.

There are several safety features with volume guarantee that ensure the tidal volume is safe and as accurate as possible.

- If the inflating tidal volume is more than 130% of the set expired tidal volume the current inflation is stopped.
- The PIP does not change, up or down, by more than 3 cm H_2O from one inflation to the next. This works independently for triggered and untriggered inflations [22].
- There is a separate control of PIP for triggered and untriggered inflations. This is because the baby is contributing to the delivered tidal volume and the PIP required with triggered inflations will be lower than with untriggered inflations where the tidal volume has to be achieved totally by the PIP.
- Sudden large changes in PIP can occur from one inflation to the next if the back-up rate is set too close to the infant's spontaneous rate because the ventilator changes from triggered to untriggered inflations with the untriggered inflations having a larger PIP than triggered ones. However, importantly it does not result in sudden large changes in tidal volume because these are being controlled. This over rides the algorithm for the PIP not changing from one inflation to the next by more than 3 cmH_2O.
- The PIP can be limited to a level the clinicians are comfortable with. However, this frequently causes the alarm "low tidal volume" when the ventilated needs to deliver a higher PIP to deliver the set tidal volume. It is important the ventilator is able to choose an appropriate PIP. A peak PIP of 35 cmH_2O is the lowest that should be set.

The expired tidal volume is used as the set tidal volume rather than inspired tidal volume because part of the inflation tidal volume is lost through leak around the ETT.

The volume guarantee automatically weans the PIP as the lungs heal, compliance and resistance improve and the baby breathes more effectively. If a baby generates a tidal volume larger that the set tidal volume the PIP may reach the PEEP level.

When ventilating with volume guarantee I always use the A/C mode so that every spontaneous breath triggers an inflation. With this combination of A/C and volume guarantee the baby's spontaneous rate controls the ventilator rate and the volume guarantee controls the PIP. Therefore to control the $PaCO_2$ the important control is the set tidal volume. This can be increased to reduce the $PaCO_2$ or lowered to increase the $PaCO_2$.

68.6.6 Pressure Support Ventilation

With the Drager 8000+ ventilator there is another triggered mode called pressure support ventilation (PSV). This is a confusing term because all the other modes are pressure supported. It is a mode that triggers expiration and therefore automatically controls the inflation time. The ventilator tracks the inflation flow curve as it rises and falls. As the flow decreases from the peak flow to 15% of the peak flow this triggers the ventilator to stop inflation. It automatically takes account of any leak flow. It is therefore important the expiratory time is set much longer than normal ventilation to allow the ventilator to increase the expiratory time if needed. An expiratory time of 0.6 sec would be appropriate even though the program will usually be choosing expiratory times nearer 0.3 sec.

The shape and timing of the inflation flow curve is dependant on the gas flow in the circuit. A high gas flow will result is a shorter inflation time and a low gas flow a longer inflation time. When ventilating a baby with a low gas flow e.g., 4 L/min then the pressure support mode is very valuable to pick the appropriate expiratory time. The advantage of this mode is that the infant cannot exhale against inflation.

68.6.7 High Frequency Ventilation

This mode of ventilation is used when "conventional" ventilation is unable to ensure satisfactory CO_2 exchange without using a high PIP (e.g., 30 cmH_2O or more) to deliver the tidal volume.

High frequency ventilation (HFOV) is done in different ways by different ventilators and so I will concentrate on the principles.

- The lung volume is produced and maintained by a continuous high mean airway pressure ~10–20 cmH_2O, not just an end expiratory pressure of ~5 cmH_2O.
- The tidal volume delivered is approximately equal to the infants dead space volume ~2 mL/kg.
- The ventilator rate is much higher than conventional ventilation at between 5 and 15 Hz (300–900 /min).

Adjusting three main parameters controls the ventilation:

1. The mean airway pressure (MAP). As this controls the lung volume and oxygenation is approximately proportional to lung volume the MAP is increased until the FiO_2 required to produce an appropriate PaO_2, is at the lowest that can be achieved. If the MAP is increased too much the oxygenation will deteriorate. It is best to start with a MAP slightly higher than that used on conventional ventilation and increase it until FiO_2 is optimized. Frequent chest X-rays will provide some information about the lung inflation, particularly whether the lungs are under inflated, one lung is collapsed, the lungs are seriously over inflated or there is air leak. Changing MAP has the same effect on all HFOV ventilators.
2. The amplitude of the ventilating pressure swing (ΔP). This is the change in the pressure that is driving the change in tidal volume. This is one controller of CO_2. The higher the ΔP the more CO_2 is removed. The best way to judge whether the ΔP is correct is to start low and increase it until the chest is seen to be just wriggling with the ventilator. It is very easy to quickly over-ventilate babies with HFOV and drive the $PaCO_2$ down to dangerous levels in a few minutes. To monitor, use a transcutaneous CO_2 sensor before HFOV is started. As the $PaCO_2$ falls ΔP is reduced accordingly. The ΔP is not very powerful with the Drager 8000+ and increasing it above 60% has little added benefit.
3. The high frequency rate also affects the $PaCO_2$. It is important to realize this is the opposite of conventional ventilation: as the rate is reduced the $PaCO_2$ is likely to fall and as the rate is increased the $PaCO_2$ rises. This is because at a lower rate the ΔP has more time and therefore is likely to increase removing more CO_2, as the rate increases the ΔP is reduced. It is best to start at ~ 8–10 Hz, adjust the MAP and ΔP to optimize the blood gases and only adjust the frequency if the $PaCO_2$ cannot be controlled at the frequency chosen. With the Drager Babylog 8000+ changing the rate has more effect on $PaCO_2$ than changing ΔP.

The advantages of HFOV are:

- Improvement in oxygenation by using a higher mean airway pressure than can be used in conventional ventilation modes.
- Better CO_2 removal in infants with very stiff lungs where a high PIP would be required to produce an appropriate tidal volume.

The disadvantages of HFOV are:

- It does not work with the baby's spontaneous intense breathing.
- The Sensormedics ventilator provides no feed-back to the clinicians.
- The Sensormedics ventilator has few alarms about the adequacy of ventilation. In fact the ETT can be clamped and no gas delivered to the baby and yet there is no alarm.
- It is very easy to over ventilate and produce hypocarbia with HFOV.
- It is not clear how well the infant is breathing or when the infant is ready for weaning.

68.7 Weaning the Infant from Ventilation

The longer the baby is intubated and ventilated the more likely infection will occur and neonatal chronic lung disease develop. It is therefore important to wean babies from the ventilator and extubate them as soon as possible.

The FiO_2 is reduced to maintain appropriate PaO_2 and SpO_2. The PIP (tidal volume) is reduced to maintain the $PaCO_2$ in the normal range.

When the FiO_2 and PIP have been reduced to ~ <0.40 and <16 cmH_2O respectively the lungs are improving and if the baby breathes adequately extubation may be possible.

Some people advocate reducing the ventilator rate in SIMV mode so that the baby has more spontaneous and less supported breaths. One problem is the unsupported breaths are made through the ETT and are ETT CPAP. This increases the work of breathing. This becomes significant if the ventilator rate is reduced below about 30/min. Lower rates are unnecessary because to maintain a normal $PaCO_2$ the baby will be breathing well.

Many babies can be extubated from A/C ventilation when the FiO_2 and PIP are low without the need for SIMV. Similarly, babies can be extubated from SIMV at 30/min.

To help decide whether a baby will breathe adequately after extubation the ventilator can be switched to ETT CPAP for ~ three minutes, and see how well the heart rate, SpO_2 and respiratory pattern are maintained [19]. A baby who can maintain the SpO_2 and heart rate on a few minutes ETT CPAP has a high chance of successful extubation. A baby who becomes bradycardic or hypoxic during this time is unlikely to breathe well after extubation.

Preterm babies should be treated with caffeine [31, 32] or theophylline before extubation. This increases the chance of

successful extubation. They should be extubated to nasal CPAP rather than straight to unsupported breathing. This has been shown to reduce the incidence of reintubation [33].

68.8 Managing Complications of Ventilation

68.8.1 Endotracheal Tube not in the Trachea

In order for a baby to be ventilated an ETT has to be passed into the trachea. This is not a simple procedure and it has been shown that this takes two or more attempts [34, 35]. It has also been shown that the person intubating the baby often thinks (hopes) that the ETT is in the trachea and it may take several minutes before they realize that the baby's failure to respond to the ventilation is because the ETT is not in the trachea. Some infants have been seriously compromised because the clinicians failed to appreciate they had intubated the esophagus. It is so important to ensure the ETT is in the trachea that after every intubation a CO_2 detector [36] or a gas flow detector are connected to the ETT so that expired CO_2 or the inspiratory and expiratory gas flow can be detected to ensure the ETT is properly placed.

68.8.2 Endotracheal Tube Inserted too far down into the Right Main Bronchus

If the ETT is either inserted too far, or it slips down after insertion, it will enter the right main bronchus. The left lung and right upper lobe will then not be ventilated. The diagnosis is made from a chest X-ray. The treatment is to withdraw the ETT to the appropriate level.

68.8.3 Accidental Extubation

Accidental extubation of a ventilated baby is a serious problem because the baby's condition may deteriorate quickly. Securing the ETT so it does not come loose can prevent it. Accidental extubation can be difficult to differentiate from other causes of a rapid deterioration. There are several ways to make the diagnosis. The quickest, most accurate technique is to watch the display of the ETT flow wave. If the ETT becomes dislodged the flow pattern immediately changes so there is flow down the ETT but not back up. Placing a carbon dioxide detector on the distal end of the ETT will also rapidly show whether CO_2 containing gas is coming out of the ETT [37]. This is rapid and sensitive. Other techniques, such as auscultation of both lungs and misting of the ETT are not so accurate. Inspection of the larynx with a laryngoscope is invasive but the only way to determine the position of the ETT if other methods are unavailable.

68.8.4 Pneumothorax

A tension pneumothorax is one of the commonest causes of acute deterioration in a ventilated baby. This is manifest by an increased FiO_2 and/or a rising $PaCO_2$ and increased ventilatory pressures when in volume guarantee mode. Occasionally it presents with apnea.

The diagnosis is made by seeing a pneumothorax on an urgent chest X-ray. Occasionally, the pneumothorax is not very obvious because the gas is anterior and with the baby lying supine the lung appears to fill the chest. One of the best radiological signs is an obvious clear demarcation around the heart, diaphragm or mediastinum. These are normally blurred in a baby with RDS. Transillumination of the chest with a bright light in darkened surroundings usually shows the hemithorax glowing. Although this is useful in an emergency it is not completely reliable and so a chest X-ray should always be obtained to confirm the diagnosis if possible.

The treatment is an intercostal drain, placed under strict sterile conditions, with the distal tubing underwater in a sealed bottle with suction applied to the outlet. The insertion site should be the 4th or 5th intercostal place in the anterior axillary line but away from the breast bud. The baby should be placed on his back with the affected side elevated so the body is at approximately 45° to the mattress. The drain is then inserted from the posterior lateral aspect. This ensures the drain passes anteriorly rather than posteriorly and therefore drains the air at the front of the chest. Under local anesthetic a small hole is made in the chest wall, just above a rib, and then enlarged appropriately with small artery forceps. These are used to introduce the catheter. A trocar should not be used because this increases the chance of perforating the lung.

68.8.5 Pulmonary Interstitial Emphysema

This mostly occurs in ventilated very premature babies. It can be a serious problem and may cause death if not treated promptly and effectively. In this condition gas tracks into the interstitial tissues and compresses the airways. It commonly occurs throughout one or both lungs and probably comes from a tear in the hilum where the gas is forced into the tissues. It causes a serious deterioration in the respiratory and clinical status of the baby. The diagnosis is made from the chest X-ray.

There are two important aspects to the treatment of PIE [38]. 1) Reduce the ventilator pressure, even if the $PaCO_2$ or FiO_2 rise as a consequence. However, do not compromise the baby's clinical and physiological stability to an extent where the baby may deteriorate. 2) Lay the baby with the affected side down and with the back at right angles to the mattress. The affected lung is dependant and slowly looses the PIE.

This is one of the best ways of treating PIE. If both lungs are effected, lay the baby so the worst lung is dependent. This treatment may lead to complete collapse of the dependent lung. This is a transient problem and with repositioning the lung usually reinflates without PIE.

68.9 Drug Treatments During Ventilation

68.9.1 Muscle Relaxing Drugs

In the past muscle relaxing drugs were commonly used for ventilated babies. This has now become uncommon, as neonatologists have realized that it is better to support the spontaneous breathing of the baby rather than take over ventilation completely. There is no evidence that the use of muscle relaxing drugs improves the outcome for babies with RDS [39]. In fact their use reduces venous return, causes edema, increases the ventilator pressure required and prolongs ventilation. They should only be used when babies are very difficult to ventilate and opposing inflation.

68.9.2 Caffeine

Caffeine when given to ventilated very premature babies has been shown to reduce the incidence of apnea, reduce the duration of ventilation, duration of oxygen treatment, the incidence of BPD and may reduce the incidence of neurodevelopmental problems [31]. It appears to have few serious side effects [32]. The beneficial effects strongly suggest that it should be given early to all ventilated very preterm infants.

68.9.3 Sedation

Many people sedate ventilated infants to make them feel less stressed and more comfortable. Several different sedatives have been used. The appropriate sedative and dose has not been elucidated. There is no good evidence that they improve the outcomes for the babies and they may adversely affect the baby's respiratory drive so that it takes longer for the baby to breathe sufficiently to be extubated [40, 41]. The best way to keep the baby comfortable is with good nursing care and appropriate positioning.

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69

Continuous Positive Airways Pressure and other Non-Invasive Ventilation Techniques

Fabrizio Sandri and Gina Ancora

69.1 Background

Continuous positive airways pressure (CPAP) is a method of assisted respiration that consists of the application of continuous positive pressure to a spontaneously breathing patient's airways throughout the entire respiratory cycle. The first use of a CPAP was in the 1930s [1–3], but its first notable application in the neonatal field was in 1971 when CPAP was used in the treatment of RDS (respiratory distress syndrome) in spontaneously-breathing newborns undergoing tracheal intubation [4].

There is a distinction between CPAP and PEEP (positive end expiratory pressure). CPAP maintains a constant pressure of a level above ambient air pressure, thus providing a continuous transpulmonary pressure gradient during the various phases of the respiratory cycle. Another term for CPAP is continuous distending pressure [5]. Administered nasally, (a technique first proposed in journals by Italian authors in the early 70s [6, 7]) CPAP became a cornerstone of respiratory support in the 1990s. When PEEP is used, positive pressure is applied to the airways of a patient undergoing mechanical ventilation in the phases between artificial breaths.

69.2 Physiopathology

69.2.1 Respiratory System

The main purpose of CPAP in the newborn is to reduce the work of breathing (WOB). Work is expressed as the function of force × distance and specifically, in the case of respiratory work, as pressure × volume ($P \times V$). It is necessary to create a pressure gradient between the atmosphere and the alveoli so that a volume of air comes into contact with the alveolar-capillary membrane during each inspiration. The pressure generated must be capable of overcoming the elastic properties and resistance of the respiratory system, thus

$$P_{tot} = P_{el} + P_{res} = V/C + FR$$

where P_{tot} is the total pressure, P_{el} is the pressure necessary to overcome the elastic forces, P_{res} is the pressure required to overcome the resistance, C is compliance, R is resistance, V is volume and F is flow (equation of motion of the respiratory system).

The amount of work expended in order to expand the ribcage depends principally on:

- the elasticity of the lungs (the work is inversely proportional to the compliance of the lungs);
- the resistance to airflow imposed by the airways;
- the elastic and resistive properties of the ribcage.

We will examine below the physiopathological characteristics of the neonatal respiratory system, with particular attention to aspects relating to premature infants and will analyze the effects of CPAP application with respect to neonatal respiratory physiology.

The neonate, particularly if premature, has a double disadvantage in performing respiratory work:

- there is increased pulmonary elastic resistance;
- the airways provide considerable resistance;
- the compliance of the ribcage is increased.

69.2.1.1 Increased Pulmonary Elastic Resistance

The lungs are elastic in nature – elasticity describes the tendency of a structure to return to its initial state after the application of a force causes it to depart from its initial conditions. Compliance (the change in volume created by change in pressure: $\Delta V/\Delta P$) is the inverse of elasticity and measures the distensibility of a structure.

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Premature newborns present with reduced compliance for various reasons:

- low surfactant production with consequent increase in pulmonary surface tension and reduction in pulmonary volume at the end of expiration (functional residual capacity, FRC);
- excessive quantity of pulmonary fluid: the clearance of the pulmonary fluid is slower in the premature newborn, particularly when delivered by elective cesarean section. Furthermore, the left to right shunt that occurs when there is a patent ductus arteriosus, especially during the recovery phase of RDS, can contribute to an increase in pulmonary fluid.

69.2.1.2 Greater Resistance of the Airways

The premature newborn presents increased resistance above all due to the proximal airways for various reasons:

- absence of the superficial layer of adipose tissue in the neck that helps to stabilize the airways, thus keeping them patent [8, 9];
- reduced mobility of the genioglossus muscle that normally stabilizes the pharynx [10];
- reduced diameter of the airways;
- increased compliance of the airways, which creates a tendency for them to collapse during inspiration; this increased compliance also increases the ventilation of the dead space.

The premature newborn also presents increased resistance at the level of the peripheral airways. The reasons for this are:

- the structural composition of the lungs is insufficient to keep the smaller airways open;
- the reduction of FRC causes a reduction in diameter of the smaller airways.

69.2.1.3 Increased Compliance of the Ribcage

Compliance of the ribcage is inversely proportional to gestational age. The compliance of the ribcage is around 5 times greater than that of the lungs in premature newborns (for normal values of pulmonary compliance) and around 3 times greater in full-term newborns. In adults the ratio is 1:1 [11]. As such, the newborn's ribcage is much weaker and more flexible, and is therefore incapable of maintaining adequate transpulmonary pressure at the end of expiration. This brings a reduction in FRC, ventilation at near-closing volumes, alveolar collapse, imbalance in the ratio of ventilation to perfusion and hypoxemia (see Chapter 57).

The combined effect of reduced pulmonary compliance and increased compliance of the ribcage results in a reduced FRC (Fig. 69.1) with a tendency towards atelectasis and a reduction in gaseous exchange.

A mechanism enacted by the newborn to maintain an adequate FRC is the partial closure of the epiglottis during expiration (grunting): the premature newborn often cannot maintain adequate laryngeal tone and so may undergo a loss of pulmonary volume. It has been shown that the suppression of grunt caused by endotracheal intubation creates a reduction in paO_2 [12].

Aside from the cry, the newborn has two further mechanisms to increase FRC: 1) Maintaining the contraction of the inspiratory muscles during the first phase of expiration; 2) commencing the inspiration before expiration has finished. This strategy, which has the effect of increasing the FRC (dynamic increase of FRC), can facilitate gaseous exchange but is disadvantageous from the point of view of the amount of energy expended [13]. Fatigue or the presence of apnea in the newborn with respiratory distress syndrome can defeat the above-mentioned mechanism and can encourage the development of atelectasis with consequent respiratory insufficiency [5].

The reduction in FRC means that the newborn is working in the lower part of the compliance curve (Fig. 69.2, part A) and so is constrained to develop high pressures in order to gain small increases in volume. A low volume at the end of inspiration also induces closure of the small airways, followed by collapse of the alveoli. This necessitates greater pressures during inspiration in order to reopen the collapsed alveoli (critical opening pressure). Pulmonary collapse also causes epithelial damage with consequent protein exudates and surfactant consumption (see Chapter 70). This reduction in pulmonary volume also brings a change in the ratio of

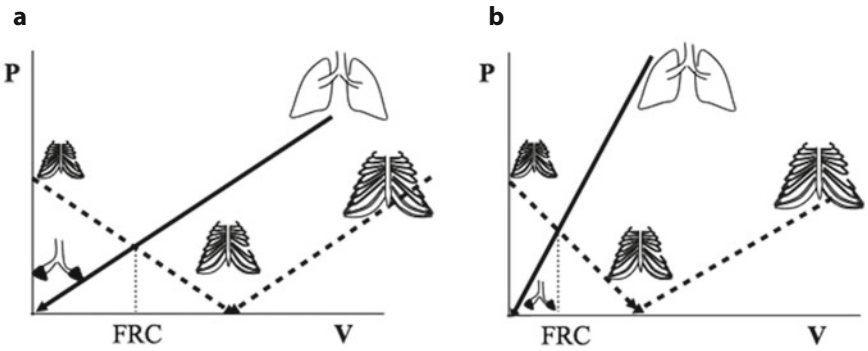

Fig. 69.1 Schematic representation of lung elastic (*continuous line*) and of chest wall (*discontinuous line*) forces in static conditions: lung tends to a volume equal to zero whereas the chest wall tends to a volume different from zero: the functional residual capacity (FRC) is achieved when the retraction force of the lung is equal to the expanding force of the chest wall. FRC is lower in the preterm (**b**) compared to the term newborn (**a**) due to both an higher force of lung retraction (lower lung compliance) and to a lower force of chest wall expansion. *P* pressure, *V* volume

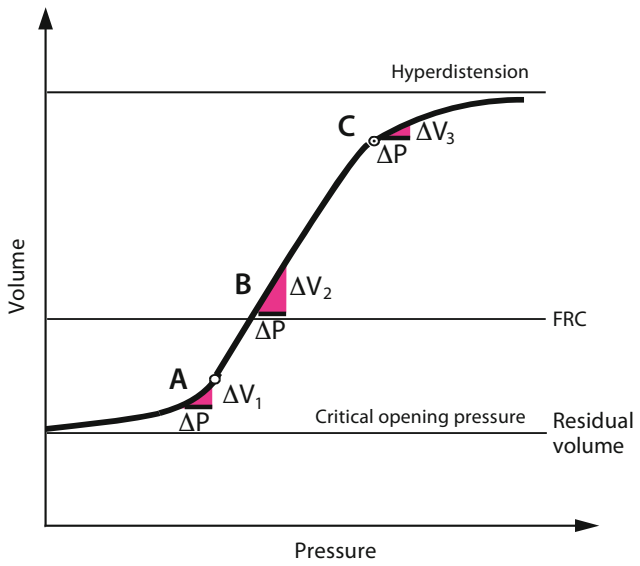

Fig. 69.2 Pressure/volume curve of the lung (compliance curve). ΔV = change in volume; ΔP = change in pressure; FRC = functional residual capacity. Normal compliance in B; low compliance in A and C

ventilation to perfusion with a consequent increase in the alveolar-arterial gradient and CO_2 concentration.

69.2.1.4 Respiratory Effects of CPAP

The application of continuous positive pressure to the airways amplifies the effect of the strategies by the spontaneously-breathing newborn that are aimed at maintaining an adequate volume at the end of expiration, minimizing the energy expended to stabilize the respiratory system, and carrying out the mechanical work of respiration. This prevents collapse of the airways and improves many critical situations that bring about an increase in the respiratory workload required of the newborn. In summary, CPAP acts on the respiratory system by the following mechanisms.

1. Increasing FRC (Fig. 69.2, Part B), eliminating dynamic elevation of FRC reducing resistance in the airways. The reduction in airways resistance is obtained by:
   - increasing the transverse section of the pharynx (the oropharynx can account for 66% of supraglottidal resistance [14])
   - dilating the airways and making them more rigid, stabilizing them and preventing their collapse. In particular an increase from 12.5% to 47% of the ratio of width to length of the larynx at the moment of maximum abduction of the vocal cords has been shown following CPAP application [15]
   - increasing the FRC thus can favor caudal traction across the supraglottidal airways reducing their resistance [16].
2. Reducing the R-L shunt thanks to the improved oxygenation and the consequent vasodilation of the pulmonary circulation.
3. Stabilizing the thoracic wall, reducing its distortion and curbing paradoxical movement.
4. Regularizing and slowing respiratory frequency [17].
5. Improving the capacity to generate a valid inspiratory force and thus to reopen collapsed alveoli following an obstructive event, probably through the elimination of the Hering-Breuer deflation reflex which is activated by a distortion in the inferior portion of the ribcage [18]. In this way it also reduces the incidence of obstructive apnea.
6. Increasing the average pressure in the airways (mean airways pressure–MAP) increasing the ventilation-perfusion ratio.
7. Preserving the surfactant on the surface of the alveoli
8. Reducing alveolar edema.

Normalizing pulmonary volume, ventilations/minute and the ventilation-perfusion ratio, CPAP improves oxygenation, increases the elimination of CO_2 and reduces the WOB [1].

69.2.2 Cardio-Circulatory System

CPAP results in a reduced preload in the right ventricle and consequently systolic output. The extent of these effects depends on how much pressure is applied by the lungs to the vascular spaces: the lower the compliance, the lower the applied pressure [19]. In the premature newborn, in the presence of a highly-compliant ribcage and often low pulmonary compliance, the pressure applied by the airways to the pleural spaces is only 5–10%, with little effect on cardiac output. In full-term newborns with normal lungs, 25% of the pressure in the airways (Paw) can be transferred to the pleural spaces and therefore the use of high pressure in these babies can have a significant effect on cardiac output. The increased thoracic compliance of the premature newborn therefore protects the intrathoracic vascular structure from a large increase in intrapleural pressure and from a consequent reduction in central venous return [11]. On the other hand, CPAP reduces pulmonary resistance in babies with RDS because of the vasodilatation of the pulmonary circulation resulting from improved oxygenation; this is the most important effect in newborns with respiratory pathologies treated with CPAP.

69.2.3 Renal Function

Renal function depends largely on the hemodynamic situation of the patient [20]. In cases of low pulmonary compliance, the application of CPAP brings an improvement in oxygenation and renal function can only be positively affected. In the presence of restricted water intake for the treatment or prevention of a patent ductus arteriosus – a common situation in cases which require the application of CPAP – the administration of dopamine at renal doses may be useful (approx. 3 μ/kg/min).

69.2.4 Intracranial Pressure

A reduction in cerebral perfusion has been reported in the course of the application of PEEP. This is related to the level of the PEEP.

An increase in intracranial pressure was found during the application of CPAP by a box around the head, due to compression of the veins in the neck. It does not appear that CPAP applied by endotracheal or nasal tube results in an increase of intracranial pressure [20].

69.3 Technical Aspect

CPAP can be delivered by a variety of devices and interfaces. There are two main types of CPAP devices, categorized on the basis of the flow characteristics: continuous and variable flow systems.

An important prerequisite for success in the use of CPAP is a circuit that allows the pressure to remain at the desired level throughout the respiratory cycle. Excessive inspiratory or expiratory fluctuations directly increase WOB and offset any benefits in pulmonary mechanics produced by CPAP [21].

Substantial differences were found in the fluctuations of Paw in different respiratory circuits hooked up to a mechanical lung model [22].

69.3.1 Continuous Flow Systems

These systems use a resistance to flow at the end of the respiratory circuit to produce pressure above that of atmospheric pressure. These CPAP systems include bubble CPAP. This device uses a fixed gas flow, and the column of water in the expiratory limb generates CPAP equal to the length of the tube that is immersed under water, which creates chest vibrations through bubbling. All these devices are also fitted with systems to humidify and heat the air. Continuous-flow mechanical ventilators can also be used to produce CPAP by the means of the PEEP valve to maintain continuous positive pressure throughout the entire respiratory cycle.

69.3.2 Variable Flow Systems

69.3.2.1 Benveniste System

The positive pressure is generated by a jet of gas directed toward an opening into a small chamber from which the mixture for the newborn comes out. Flow rates of 4–20 L/min serve to generate pressures in the chamber from 0–13 cmH_2O. These pressures are comparable to those measured at the oropharynx in the course of CPAP administered via 1 or 2 nasal prongs (this is indifferent, as long as the mouth is kept closed by use of a pacifier) [23].

69.3.2.2 Infant Flow System (Fig. 69.3)

A generator is connected to a circuit composed of a 3-channel tube (Fig. 69.4): one channel (inspiratory limb) provides gas flow and is connected to the nasal cavity via nasal prongs or mask, one (expiratory limb) is open to ambient pressure and one is used to measure the level of pressure created at the generator level. The flow provided by the Infant Flow driver is accelerated in the twin injector nozzles of the nCPAP generator. When the patient makes a spontaneous inspiratory breathing effort, the generator provides assistance by converting the kinetic energy of the flow to pressure energy. When the infant makes a spontaneous expiratory effort a pressure is applied at the nasal attachment of the NCPAP generator. This causes the flow to flip round and leave the generator via the expiratory limb. CPAP is maintained at the nasal connection throughout. When the expiratory breathing effort stops, the flow instantly flips back to the inspiratory position. The operating principle is that the high-pressure jet flows in different directions in response to the pressure generated at the level of the nasal cavities and the respiratory effort of the patient. During inspiration the drop in pressure in the nasal cavities creates a pressure gradient that directs the flow toward the nasal cavities, thus assisting inspiration. During expiration there is an inversion of the direction of the jet that helps the expiration (Coanda effect). The kinetic energy of this high-velocity flow is transformed into pressure in the area of the nasal cavities thus generating CPAP. Flow rates between 5 and 11 L/min generate pressures between 2 and 10 cmH_20. As such, the system does not require an expiratory valve. Tests on mechanical lung models have demonstrated that a CPAP circuit achieves greater stability in Paw and a reduction in WOB of around one quarter compared to traditional circuits [24–26]. Available clinical studies have, on the other hand, reported conflicting

Fig. 69.3 Infant flow generator

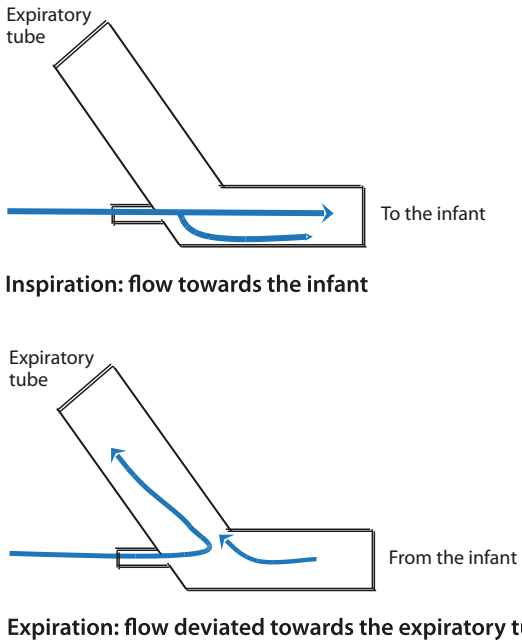

Fig. 69.4 Functioning of the infant flow generator

results. Two studies did not show any difference between this system and traditional ones in terms of improving pulmonary compliance, reducing oxygen requirements and improving respiratory frequency [27, 28].

A recent study comparing 3 circuits (Infant Flow system, double nasal prongs and single nasal prong fed by mechanical ventilators) for the application of NCPAP in newborns weighing less than 1800 g with light respiratory distress or apnea demonstrated better pulmonary recruitment using the Infant Flow circuit [29].

69.4 Methods of Administering CPAP

- *Endotracheal Tube* This method entails a notable resistance (directly proportional to its length and inversely proportional to its radius) and as such involves a significant increase in WOB. At this time it is no longer recommended as a system for the administration of CPAP. Furthermore, by bypassing the nasal and pharyngeal mucosa, it creates an alteration in the humidification and heating of the inhaled gases. Also the trauma applied to the respiratory mucosa makes its use inadvisable for newborns who do not need mechanical ventilation.
- *Head-Box (Helmet CPAP)* This system is of low efficacy below 1500 g bodyweight, extremely noisy and cumbersome and does not allow access to the newborn's head. Furthermore, it has been associated with an increased incidence of cerebral hemorrhage due to compression of the neck [30]. Aspiration pneumonia has also been reported during feeding.
- *Face Mask* This system too is rarely used, particularly for premature infants. The mask should cover the nose and mouth and should always be used with an orogastric feeding tube to avoid excessive abdominal distension. In the premature newborn, apart from the problem of keeping the mask in place and the consequent difficulty of generating a sufficient level of CPAP with adequate flow, there are also problems of excessive dead space that can be 2 or 3 times greater than in a full-term newborn. Cases of cerebral hemorrhaging have been reported in very low birth weight newborns undergoing this therapy due to the forces applied to the back of the head by the mask's attachments [31].
- *Facial Chamber* The entire face protrudes into a chamber held in place by a latex ring filled with styrene particles. This system too is now not used as it does not permit access to the newborn's face for alimentation or aspiration.
- *Nasopharyngeal Tube (Mono-Nasal CPAP)* The same type of tube used for tracheal intubation is inserted through the nostril as far as the pharynx. This is a simple technique, which is widely used for mild forms of respiratory distress or apnea in the premature. The disadvantages lie in the fact that this tube provides elevated resistance, particularly if it is of small diameter, and that the tube also totally bypasses the humidifying-heating action provided by the nasopharyngeal mucous.
- *Nasal Prongs (Bi-Nasal CPAP)* Currently this is the most used and effective system for the administration of CPAP in the newborn. The baby has to breathe through the nose; the nasal prongs also allow the air to be humidified and heated by most of the nasopharyngeal mucous. The mouth in this case acts as a pop-off pressure valve. The nasal prongs are easily applied and less invasive than the endotracheal tube; first examples of nasal prongs were put into use in the early 1970s [6, 7, 32]. Particular attention has been paid to the work required of the newborn in order to overcome the resistance due to the small diameter of the nasal prongs (the so-called superimposed work of breathing). A study reported an increase of 100% in WOB during CPAP administered with nasal prongs compared to CPAP with a face mask [33]. A closer evaluation of the study, however, revealed that this increase could, in part, be put down to the increased resistance caused by the catheter placed inside the prongs to relieve pressure. Recently these prongs have been redesigned with a precise angle and structure in order to minimize resistance to the airflow [26]. Another problem with these prongs is related to the loss of flow (and hence of pressure) around the prongs themselves if they are not secured properly. Nowadays the prongs are made from a soft silicon material that expands slightly in contact with the warmth and humidity of the nostrils, adapting to their dimensions and so favoring a good fit.
- *Nasal Masks* These devices, which consist of silicone masks that completely cover the nose, are sometimes a useful alternative to nasal prongs, in particular in extremely low birth weight infants.

69.5 Clinical Applications

69.5.1 Respiratory Distress Syndrome-RDS

The main physiopathological aspects of RDS, whose principal cause is a deficit of surfactant [34], are: reduction in pulmonary compliance; reduction in FRC and alteration in the ratio of ventilation to perfusion. The rigidity of the ribcage is also reduced with a paradoxical distortion during inspiration which is greater the larger the reduction in pulmonary compliance, with a consequent reduction in tidal volume [5, 35]. These alterations lead to a profound alteration in gas exchange and increase the respiratory workload, the consequences of which are the presence of hypoxemia, hypercapnia and acidosis.

69.5.1.1 Clinical Evidence

The first clinical experience of neonatal CPAP application came in 1971 with a case report of 20 patients affected by RDS [5]. This was followed by others [36–38].

The rationale behind the use of early NCAP in the VLBW newborn lies in the facilitation of the acquisition and maintenance of an adequate pulmonary volume (FRC). As well as improved gas exchange, a normal air-liquid interface is maintained at the level of the terminal alveoli. This promotes the liberation of reserves of pulmonary surfactant, which in turn further stabilize the air spaces preventing the formation of hyaline membranes and the following atelectasis that is typical of surfactant deficiency. This should lead to a decrease in the requirement for mechanical ventilation with a reduction in its complications, in particular bronco-pulmonary dysplasia.

The main reason for the interest in adopting NCPAP, especially in very low birth weight newborns (VLBW, that is <1500 g), came from the publication of two multi-centre retrospective studies. They showed that the incidence of chronic lung disease, assessed as the percentage of VLBW survivors who were oxygen dependent at 28 days from birth, was significantly lower in centers that had been early adopters of NCPAP, despite having amongst the highest survival rates. The early adoption of NCPAP at these centers was part of a low-invasiveness strategy consisting of the tolerance of relatively high pCO_2 values, up to 60 mmHg (permissive hypercapnia), before proceeding to intubation and mechanical ventilation, and the non-use of paralyzing drugs [39, 40].

Early NCPAP was largely used in VLBW infants with recourse to intubation and mechanical ventilation only when it was held to be essential, based on well established criteria [41–44]. The common denominators of these clinical experiences are:

- intubation in the delivery room only if considered essential for cardio-pulmonary resuscitation;
- early NCPAP, i.e. application of NCPAP to all newborns within the first 30 minutes of life or at the appearance of the first signs of respiratory distress;
- intubation and, if necessary, mechanical ventilation only if one or more of the following criteria are met (criteria for failure of NCPAP): untreatable apnea, respiratory acidosis (pCO_2 >65-70 mmHg and pH <7.20) and requirement of exogenous surfactant.

In general, the success of NCPAP in treating VLBWI avoiding mechanical ventilation, is proportional to the gestational age (76% of newborns ≤26 weeks GA require ventilation compared to 43% of newborns >26 weeks) regardless the use of pre-natal prophylaxis with steroids [41].

There is a general tendency to tolerate higher pCO_2 values than in the past [39, 42, 43], and this has reduced the need for intubation. Even when mechanical ventilation is undertaken, it does not require the use of aggressive ventilation strategies in search of blood gas values that are obtained at the cost of high baro-volutrauma [41].

A study carried out on 67 newborns of <1000 g birthweight (Extremely Low Birth Weight-ELBW) who underwent early NCPAP if they were not intubated in the delivery room for primary resuscitation, demonstrated that it was not necessary to intubate and start mechanical ventilation in 73% of the newborns >28 weeks and in 32% of the newborns ≤28 weeks [45]. In another study, Finer et al [46] found that all infants of 23 weeks' gestation required intubation in the delivery room whereas only 3 of 21 (14%) infants of 27 weeks' required such intubation.

The reduction in the percentage of mechanically-ventilated newborns did not cause an increase in mortality or negative side effects such as cerebral hemorrhage [41, 43, 45, 47–49].

However, despite uncontrolled and cohort studies suggesting the efficacy of NCPAP in avoiding MV and ventilatory-induced lung injury, there are few randomized clinical trials confirming the benefit of NCPAP as the primary type of respiratory support in preterm newborns. Morley et al published the COIN trial in 2008 [50]. In this trial NCPAP was compared with intubation and ventilation on the hypothesis that the use of NCPAP shortly after birth would reduce the rates of death and bronchopulmonary dysplasia. The main eligibility criteria were a gestational age at delivery between 25 and 28 weeks and an ability to breathe at 5 minutes after birth but needing respiratory support. Infants were ineligible if they had been intubated before randomization or if they required no respiratory support or oxygen. At 5 minutes after birth, the allocated treatment was started. In infants who were assigned to receive NCPAP, this was started at a pressure of 8 cm of water with nasal prongs. They were intubated and underwent ventilation only if they had any of the following clinical signs: apnea unresponsive to stimulation and methlyxanthine treatment, an arterial pH of less than 7.25 with a partial pressure of arterial carbon dioxide ($PaCO_2$) of more than 60 mmHg (8.0 kPa), a metabolic acidosis not responsive to treatment, or treatment with more

than a 60% concentration of oxygen. Infants receiving NCPAP were treated with surfactant only after intubation. Surfactant treatment, ventilation settings, and extubation and reintubation criteria were not mandated in either group and followed local protocols. Half the infants in the NCPAP group were subsequently intubated. Infants in the NCPAP group had a better outcome at 28 days than those in the intubation group. The two groups had similar outcomes at 36 weeks' gestational age, but there was an increased incidence of pneumothorax in the NCPAP group.

There are studies of great interest that have demonstrated the efficacy of an approach that prescribes the use of early NCPAP together with the administration of surfactant in the treatment of RDS. This approach, known as the INSURE approach (INtubation-SURfactant-Extubation) is based upon the adoption of the following procedure. In a newborn undergoing NCPAP, whenever administration of surfactant is indicated, the patient is intubated, surfactant is administered in the usual doses and the patient is then placed again in NCPAP after extubation (assuming that the patient is breathing spontaneously). Mechanical ventilation is only applied when indicated by well-defined criteria of failure [51].

A multi-centre study carried out in Sweden compared the incidence of mechanical ventilation in two groups of newborns affected by moderate-to-severe RDS to whom early CPAP was applied (e.g., application at the first clinical signs of respiratory distress). The treatment group received the INSURE approach if their oxygen requirement exceeded 60%. The control group received treatment only with NCPAP and surfactant was reserved for cases where mechanical ventilation was required. The criteria for application of mechanical ventilation (apnea and or oxygen requirement >80%) was the same for both groups. Recourse to mechanical ventilation was reduced from 85% in the control group to 43% in the treatment group [52].

The same authors later carried out a multi-centre study on newborns with RDS below 30 weeks gestational age and who were undergoing early NCPAP. They demonstrated that when the INSURE approach was adopted early on in RDS, i.e. at an oxygen requirement between 40% and 60%, there was a reduction in the need for mechanical ventilation and of death in the first seven days post partum from 63% to 21% compared to a later approach (oxygen requirement >60%) [53].

This last work, aside from demonstrating the efficacy of the INSURE approach, permitted the establishment of a limit to the oxygen requirement in patients with RDS undergoing NCPAP (FiO_2 about 40%) above which the administration of surfactant is indicated.

A study conducted in Italy on 155 newborns of gestational age ≥28 and <32 weeks treated by the INSURE approach at oxygen requirements >40%, showed no difference in requirements for surfactant and mechanical ventilation comparing two groups to which NCPAP had been applied: in the first group NCPAP was applied within 30' of birth, regardless of the clinical picture; in the second group NCPAP was applied only in the presence of respiratory distress with oxygen requirements >40% [54].

A recent systematic review of the INSURE approach, reported a reduced need for MV in the first week of life, when used early in respiratory distress syndrome [55].

Either prophylactic surfactant or delivery room NCPAP to maintain functional residual volume were identified as potentially beneficial practices which, if adopted in extremely preterm infants, could reduce lung injury [56].

Recently, the CURPAP study compared the administration of prophylactic surfactant followed by NCPAP (prophylactic INSURE) with early NCPAP followed by early selective surfactant given through a brief course of endotracheal intubation (early rescue INSURE) to preterm newborns of GA 25-28 weeks not intubated at birth [57]. In both groups, MV was started after surfactant in the absence of good respiratory drive. Infants who were extubated to NCPAP after surfactant were eligible for MV if the following NCPAP failure criteria occurred: FiO_2 >0.40 on NCPAP to maintain oxygen saturation of 85–92% for at least 30 minutes unless rapid clinical deterioration occurred, intractable apnea, respiratory acidosis defined as PCO_2 >65 mmHg (8.5 kPa) and pH <7.20. This study showed that, in spontaneously breathing preterm newborns treated with NCPAP, prophylactic surfactant given within 30 minutes from birth is not superior to early selective surfactant in terms of requirement of MV in the first 5 days of life.

The main implication for clinical practice of the CURPAP study is that NCPAP should be started soon after birth in spontaneously breathing infants of 25–28 weeks' gestation and early selective surfactant should be given once signs of respiratory distress have developed. Extubation after surfactant administration should be attempted as soon as possible. With this strategy, more than 50% of infants will need only NCPAP, 49% intubation and surfactant, and nearly one third also mechanical ventilation in the first 5 days of life. Overall, the respiratory approach used in this study resulted in a very good respiratory outcome: 78–79% of infants, in both arms, survived without any supplementary oxygen or respiratory support at 36 weeks' postmenstrual age. The incidences of moderate/severe broncho-pulmonary dysplasia were 14.3 and 11.7%, respectively in the prophylactic surfactant and NCPAP groups, which is lower than the 30% incidence reported in other studies [58, 59].

In the past, doubts have been cast on the possibility of generalizing low-invasiveness treatment strategies in VLBW infants [60]. Currently, similar doubts may be raised for ELBW infants, in whom the application of results from studies [46, 50, 57] should take into account the rate of prenatal steroid use and the type of antenatal health care program.

In conclusion, the available published data suggests that:

- VLBW infants, not intubated at birth for cardiopulmonary resuscitation, can be treated conservatively using NCPAP, with the administration of surfactant and mechanical ventilation only being used if precise clinical, laboratory test and instrumental criteria are met;

- prenatal steroids, higher GA and female sex are associated with higher chances of being treated non-invasively;
- a precise limit of gestational age or birth weight below which NCPAP cannot be used with success has not yet been established;
- using NCPAP together with early selective surfactant to avoid more invasive strategies does not entail an increase in mortality rates or negative side effects.

69.5.2 Neonatal Apnea

Apnea is traditionally classified into three categories based on the presence or absence of obstruction of the upper airways: central, obstructive and mixed (see Chapter 74) [61, 62].

Because most apneic episodes have an obstructive component, CPAP appears to be one of the most effective strategies. It splints the upper airway with positive pressure and decreases the risk of pharyngeal or laryngeal obstruction. CPAP probably also improves apnea by increasing functional residual capacity (FRC) and so improving oxygenation status [61, 62]. The positive effect of CPAP is also due to the stabilization of the ribcage, [14, 16, 61], that contributes to the airways opening and to the reduction of the Hering-Breuer reflex [18].

Given that NCPAP does not have a direct effect on apnea of central origin, its use in association with stimulants of the respiratory centers such as methylxantine below 32 weeks gestational age is generally accepted [63–65].

69.5.3 Weaning the Patient from Mechanical Ventilation (Post-Extubation Phase)

It is well known that after a period (long or short) of intubation and mechanical ventilation, extubation can fail, leading to the re-intubation of the newborn [66]. The reasons for this failure are substantially as follows:

- apnea;
- an increase in oxygen requirements (this can be correlated to a loss of volume from a malfunction of the glottis after intubation, or to atelectasis);
- respiratory acidosis.

Various contributors have demonstrated the efficacy of NCPAP application in the post-extubation phase following mechanical ventilation. NCPAP compared to direct extubation reduces the need of re-intubation, both in newborns weighing < 1500 g (16% failure rate versus 52%) [67] and in those weighing <1000 g (24% failure rate versus 79%) [68]. The factors that necessitate re-intubation, principally apnea but also an increase in oxygen requirement due to loss of pulmonary volume and respiratory acidosis, are counteracted by NCPAP. A recent meta-analysis confirmed the efficacy of NCPAP in weaning of patients from mechanical ventilation and thus recommended its adoption as an elective method for use after extubation, if necessary in combination with a methylxantine [69]. To achieve successful extubation to NCPAP, it is also important to choose the most appropriate moment in terms of parameters of mechanical ventilation. This should be at a point where the respiratory problem can be considered overcome by ventilation (FiO_2 <0.35, mean airways pressure [MAP] <7 cmH_2O, ventilatory frequency ≤20/min) [67–69]. A recent study comparing a continuous flow with a variable flow CPAP device (Bubble CPAP versus Infant Flow system) concluded that both devices were effective in the post-extubation management of infants with RDS. However in infants ventilated for ≤14 days, bubble CPAP is associated with a significantly higher rate of successful extubation and with a significantly reduced duration of CPAP support [70].

69.5.4 Other Clinical Applications

- *Transient Tachypnea of the Newborn (TTN)* Some doubts have been raised regarding the safety of NCPAP in TTN (see Chapter 63) due to the theoretical risk of air trapping and pneumothorax. There are, however, no relevant published data [71]. Authoritative guidelines include TTN in indications for NCPAP application [63, 72], and our personal experience is that the early application of NCPAP for TTN brings about a reduction in the clinical signs of respiratory distress without a significant increase in the incidence of pneumothorax.
- *Meconium Aspiration Syndrome (MAS)* The application of a continuous distending pressure could be beneficial, resolving the areas of atelectasis and re-opening and stabilising the airways [20, 73]. However it should be reserved for the less severe forms of MAS (Chapter 59). More serious cases will require intubation, broncho-alveolar lavage with surfactant and mechanical ventilation [74, 75].
- *Congenital Cardiopathies with Hyperafflux* Pulmonary hyperafflux can lead to a reduction in pulmonary compliance and to a change in the ventilation-perfusion ratio, with consequent hypoxemia, which is potentially correctable with CPAP [20].
- *Tracheobronchomalacia* CPAP resolves the collapse of the airways present in this condition, considerably relieving the respiratory distress correlated with it [63, 76].
- *Atelectasis* The onset of atelectasis can be due to different factors but its resolution will require the removal of the root cause in each case. However, in many situations, e.g. post-extubation atelectasia, CPAP may be used electively [63].
- *Bronchiolitis* Early CPAP (predominantly nasal) can rapidly aid respiratory muscles, improve respiratory distress symptoms (retractions, tachypnea, agitation) and blood-gas measurements (pO_2, pH, pCO_2). These effects have

been attributed to widening of the bronchioli by positive pressure, followed by emptying of the lung and restoration of a normal FRC. This leads to a reduction in the use of mechanical ventilation [77, 78]. NCPAP has also demonstrated its efficacy in the treatment of apnea associated with RSV infection [79].

- *Paralysis of the Phrenic Nerve* Cases have been published in which paralysis of the phrenic nerve was successfully treated with NCPAP application, which corrects the deficit in transmural pressure caused by paralysis of the diaphragm [80].
- *Nebulization of Pharmaceuticals* NCPAP has recently been used for the respiratory administration of pharmaceuticals (beta blockers, steroids, acetylcysteine and adrenaline), although this does not currently seem to be of great clinical interest [81].

69.6 Side Effects

- *System Malfunctions* The obstruction of the nasal prongs or the tube by mucous (like the obstruction of the nasal passages) is common during nasal CPAP, with potentially serious consequences (sudden decrease in oxygenation) due to the lack of airflow delivered to the patient [63].
- *Pulmonary Over-Distention* Pulmonary hyperdistension is the most serious complication that can occur during NCPAP. This condition can occur whenever the positive pressure applied is excessive with respect to the patient's pulmonary compliance.
  The presence of a hyperdistended lung brings an increased risk of pneumothorax, a worsening of the ventilation-perfusion ratio and an increase in CO_2 retention and WOB. It is also known that the higher the pulmonary compliance, the greater the transmission of positive pressure to the posterior mediastinum, followed by an increase in central venous pressure with a decrease in venous return and cardiac output. Fig. 69.5 shows the effects of rising CPAP values on central venous pressure, on paO_2 and on $paCO_2$, the onset of which correlates directly with hemodynamic and respiratory interferences (Fig. 69.2, part C) [82].
  These hemodynamic effects can have serious consequences on gastro-intestinal, renal and cerebral perfusion and can cause an increase in intracranial pressure due to the decrease in venous drainage [20, 63].
- *Gastric Distension* It can cause reduced ventilatory excursion due to compression of the diaphragm or even gastric perforation (CPAP belly syndrome) [20, 63, 83]. It can be prevented by properly affixing the nasal prongs, by positioning a permanent orogastric tube and by periodically checking the degree of abdominal distension.
- *Nasal Lesions* Nasal-cutaneous lesions have been described during NCPAP with possible permanent deformities [84, 85]. These lesions can be prevented by choosing silicone nasal prongs (which permit good contact without being harmful), nasal prongs of the correct dimensions with relation to the newborn's nostrils and ensuring their correct positioning.

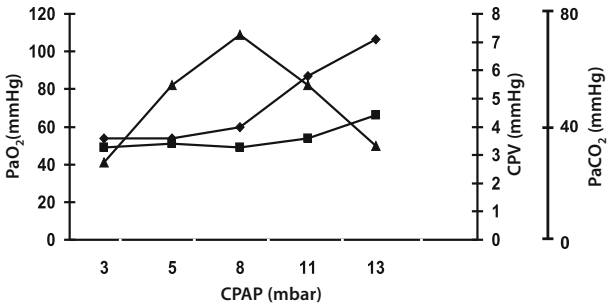

Fig. 69.5 Effects of different CPAP levels on the central venous pressure (CVP (■), PaO_2 (▲) and $PaCO_2$ (◆). Modified from [82]

69.7 Suggestions for NCPAP Use in the Clinical Practice

69.7.1 Pressure Level

There have been few data published to evaluate a method that would allow the establishment of an optimum pressure for CPAP, and its clinical applicability in practice is very limited [86, 87].

It is advisable to:

- start with a level of CPAP between 5 and 7 cmH_2O;
- individualize the CPAP level on the basis of respiratory dynamics, respiratory frequency, grunting and thoracic retraction. The level of CPAP can be increased up to 8–10 cmH_2O;
- choose higher values if there is reduced pulmonary volume;
- once an improvement in the respiratory dynamic has been reached, adjust the FiO_2 so as to maintain PaO_2 between 60 and 80 mmHg or tcO_2 saturation (measured by pulse oximetry) at between 85% and 93% in preterm infants;
- reduce the level of CPAP as long as lung compliance, lung volume and oxygenation improve in order to avoid hyperdistension and transmission of positive pressure to the mediastinum.

69.7.2 Monitoring

The use of NCPAP therapy requires accurate monitoring to both evaluate the evolution of the illness and to identify negative side effects.

- Blood gas analysis from arterial or an arterialized capillary blood, represents the gold standard for blood gases measurement. It should be carried out within 15–30 minutes of starting therapy and whenever clinically indicated [20].
- Pulse oximetry. This is the method of choice for the continuous monitoring of the level of oxygenation, particularly when the situation has stabilized, despite its universally known limitations. It is advisable to target O_2 saturation values between 85% and 93% [88].
- Transcutaneous PO_2 and PCO_2. This type of monitoring, though potentially very useful, has in reality many limitations that require great caution in interpreting the values it obtains, which should be verified and checked frequently against the blood gas values.
- ECG and systemic arterial pressure. The potential side effects of CPAP on circulation require continuous monitoring of heart rate and periodic measurement of systemic blood pressure.
- Chest X-ray provides valuable information in evaluating the effect of CPAP on the evolution of the disease, such as improvement of the radiological picture or the development of complications such as hyperdistension or pneumothorax.

69.7.3 Failure of Nasal CPAP

This subject is addressed in detail in the paragraph on clinical applications, in particular in the part dedicated to RDS.

69.7.4 Weaning from Nasal CPAP

Once clinical improvement has been obtained as previously described, the improved situation is shown by the ability to progressively reduce FiO_2 (in 2–5% steps down to 21%) and the level of positive pressure (1 cmH_2O at a time down to 2–3 cmH_2O). Once minimal values have been reached for both parameters, the NCPAP therapy can be stopped more or less gradually, having regard to the weight and gestational age of the baby and depending on the presence or lack of other indications for therapy, e.g., apnea of prematurity.

69.8 Nursing of the Neonate in NCPAP

The importance of nursing is particularly important when CPAP is administered via nasal prongs. The following require particular attention:

- correct attachment (headset and nasal prongs);
- correct positioning (circuit and infant);
- correct temperature setting on the humidifier;
- correct aspiration of the airways and feeding.

69.8.1 Attachment (Headset and Nasal Prongs)

The attachments for the nasal prongs always include a bonnet, which the prongs themselves are fixed to. The bonnet must be large enough to reach the level of the eyebrows and to cover the ears completely. If it is too small it will tend to ride up, forcing the circuit toward the neonate's nose. The prongs generally come in three sizes (small, medium and large): the size that is most compatible with the patient's nostrils must be chosen. It is wrong to assume that the smallest prongs are for the most delicate neonate. If the prongs are too small they do not give an adequate hold, which is necessary to maintain a constant level of CPAP and to avoid an increase in airways resistance. This does not allow for a reduction in WOB and may cause it to increase.

The silicone nasal prongs warm up when inserted into the nostrils, and in doing so they become softer and expand slightly, improving their hold. The use of creams or plasters around the nose is not recommended. Once the circuit has been positioned care must be taken that the edge of the prongs does not adhere to the nasal septum: the base of the prongs must always be visible (Fig. 69.6) (see also § 69.8.2).

The prongs can increase the production of nasal secretions and for this reason the system for humidifying and heating the administered gas is very important (see § 69.8.4). When the infant cries there may be a loss of pressure: therefore correct nursing is extremely important during the administration of CPAP (see also § 69.8.3).

Silicone masks are available as an alternative to nasal prongs for very small or large infants (Fig. 69.7).

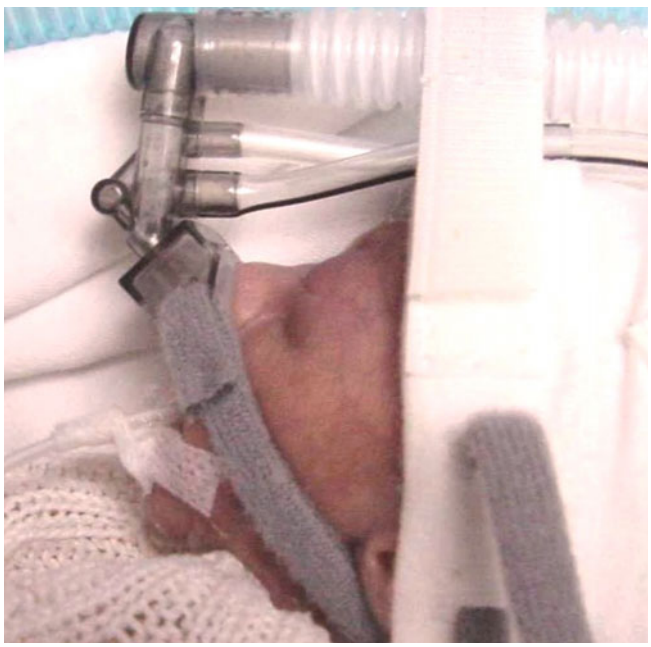

Fig. 69.6 Correct positioning of the nasal prongs

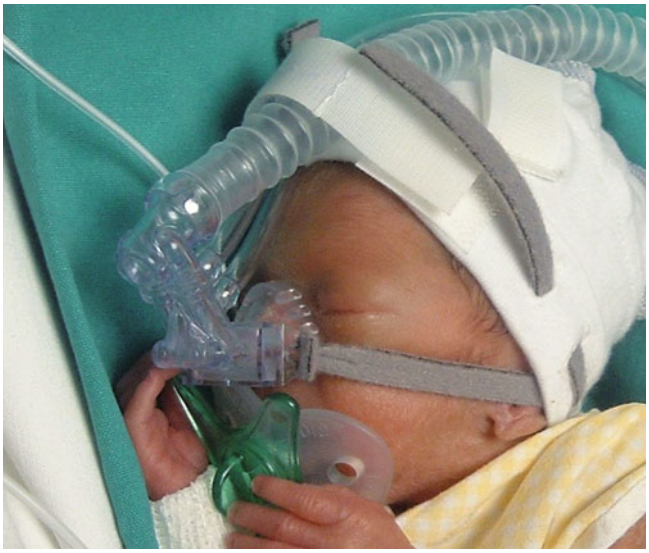

Fig. 69.7 Nasal mask for the application of nasal CPAP

69.8.2 Positioning of the Circuit

The tubes should be positioned so as not to pull on the flow generator or put pressure on the neonate's nose (Fig. 69.8)

The flow generator is connected to the nasal mask by ribbons, which must not be too tight. Once the ribbons have been fixed they should be directed downwards, that is towards the base of the ears and away from the eyes in order to avoid swelling at this level. Plasters or knots between the ribbons and the nasal mask should be avoided to facilitate the rapid adjustment of the prongs. Only the gas arrival tube should be attached to the bonnet while the others should be left free.

In the case of the Infant Flow generator it is important that the outlet tube be positioned outside the incubator in order to minimize noise: if necessary use the extension tube.

69.8.3 Positioning the Infant

The infant can be placed in a prone, supine (with support) or lateral position; the neck however must always be slightly extended. The prone position has been shown to be better for the premature neonate with respiratory distress [89].

Kangaroo-care is possible during NCPAP. It is important to keep the infant's mouth closed. This confers at least three benefits:

- it avoids loss of pressure and consequent instability of CPAP therefore reducing WOB;
- it avoids drying of the mucous and the formation of dense, whitish secretions because it favors the deglutition of saliva;
- it keeps the jaw in the forward position, thus avoiding the tongue falling backwards and guaranteeing the patency of the airways.

The infant can be helped to keep their mouth closed. Initially it can be of use to place a support (e.g., roll of gauze) under the neonate's chin. The use of a pacifier favors deglutition and reduces the formation of saliva in the infant's mouth. It also helps keep the jaw in the forward position.

69.8.4 Humidification and Heating

Adequate humidification is important in order to avoid drying of the mucous and the accumulation of dense secretions, which can block the airways. The recommended gas temperature is 37°C: this is obtained by setting the resistance temperature of the circuit at 39°C and that of the gas at 2°C less. It is always important to ensure that the temperature sensor is placed outside the incubator. If the neonate is positioned under an infant warmer, the sensor should be thermally isolated.

69.8.5 Airways Suction

It is not necessary to regularly suction the infant in NCPAP and this maneuver should be performed only when necessary. To reduce the formation of oral and nasal secretions, the following measures can be used:

- set the humidifier to a suitable temperature (see previous paragraph): if the temperature is too low it can favor the formation of dry secretions;
- keep the infant's mouth closed (see § 69.8.3).

69.8.6 Feeding

The use of NCPAP does not prevent feeding. Food intake should be encouraged (via orogastric feeding tube or bottle). Additionally, fed newborns will avoid swallowing air thus reducing abdominal distension. An orogastric tube is not always necessary.

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70

Lung Diseases: Surfactant Replacement Therapy

Henry L. Halliday

70.1 Introduction

In the past 3 decades introduction of prenatal steroid treatment, postnatal surfactant therapy and assisted ventilation have lead directly to improved neonatal outcomes [1]. Improved survival is directly related to more effective prevention or treatment of respiratory distress syndrome (RDS), which prior to the 1990s had a high mortality. Following unsuccessful clinical trials with nebulized synthetic surfactants, comprised of phospholipids without surfactant proteins, in the 1960s [2] a number of randomized controlled trials in the 1980s demonstrated benefits of surfactants instilled directly into the lungs of preterm infants [3–5]. These surfactants were of 2 main types: natural (derived from animal lungs or human amniotic fluid) [3] containing surfactant proteins-B and C (SP-B and SP-C) and synthetic [4, 5] (containing phospholipids and other agents to facilitate spreading and adsorption). Both types of surfactant given, either prophylactically (in the delivery room within 15 minutes of birth) [3, 4] or for treatment of RDS [5], increased neonatal survival and reduced pulmonary air leaks such as pneumothoraces and pulmonary interstitial emphysema.

More recently, further randomized clinical trials helped determine the best surfactant, the optimal timing for initial treatment, need for redosing and dose of phospholipids needed for best outcomes [1, 2]. Results from these trials have provided guidelines for best practice and these are now available in Europe [6], USA [7], Canada [8] and UK [9]. The aim of this chapter is to summarize the recommendations for surfactant treatment based on evidence from clinical trials and systematic reviews and to point out areas where controversy still exists.

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70.2 Respiratory Distress Syndrome and Rationale for Surfactant Therapy

Respiratory distress syndrome (RDS) is caused by surfactant insufficiency [10] which leads to progressive hypoxia, respiratory failure and mixed respiratory and metabolic acidosis as a result of alveolar collapse and increased work of breathing [11]. Surfactant deficiency is strongly associated with prematurity and progressive acidosis further reduces surfactant production. The normal surfactant phospholipid pool size at birth is about 100 mg/kg but in preterm infants this is often reduced to below 25 mg/kg and, if severe RDS is present, less than 5 mg/kg [11]. Surfactant may also be inactivated by leaking of inhibitory proteins from the plasma into the alveoli. Prior to the introduction of surfactant therapy, infants with RDS who survived began to produce their own endogenous surfactant after 2–3 days and this heralded their recovery [11]. Those infants who did not survive either developed progressive respiratory failure with irreversible hypoxic injury to the cardiovascular and central nervous systems or acute deterioration associated with pulmonary air leaks or intraventricular hemorrhage.

Surfactant therapy prevents or overcomes alveolar collapse, especially at end expiration, increases lung volumes and pulmonary compliance, and reverses respiratory failure [11]. The result is improved survival and significant reduction in pulmonary air leaks following surfactant treatment [3–5]. There is evidence from both animal studies and clinical trials that natural surfactants, containing SP-B and SP-C act more rapidly than synthetic preparations containing mainly phospholipids leading to improved outcomes with regard to survival and pulmonary leaks [12]. Timing of surfactant therapy may also be important in determining outcome with evidence pointing to earlier or prophylactic therapy being superior to later treatment [13]. It is important to note that incidence of RDS varies from about 80% at 28 weeks' gestation or below to about 50% at 30 weeks, 30% at 32 weeks and 10% at 34 weeks [11].

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

70.3 Results from Randomized Trials and Systematic Reviews

These are discussed under the following sub-headings: efficacy, timing of first dose, size of first dose, method of administration, need for redosing, type of surfactant, concomitant interventions and other indications for surfactant therapy.

70.3.1 Efficacy

Randomized clinical trials of synthetic (protein-free) and natural (containing SP-B and SP-C) surfactants, given both prophylactically (within 10 to 15 minutes of birth in the delivery room) or as treatment for established RDS in the neonatal unit, reduce neonatal mortality and pulmonary air leaks such as pneumothoraces [1, 3-5]. Although these trials took place mainly in the 1980s and 1990s, at a time when prenatal corticosteroid treatment was much less than today, there is no reason to believe that surfactant treatment does not continue to have a positive impact on the outcome of preterm infants [1, 2]. Indeed prenatal corticosteroids and postnatal surfactant have synergistic effects [14] and both should be considered when preterm birth is likely. The sizes of the effects of prophylactic surfactant administration on neonatal mortality, pneumothorax and other selected outcomes are shown in Table 70.1.

70.3.2 Timing of First Dose

It is clear that the earlier surfactant is given in the course of RDS the better the outcomes [15]. For infants of less than 31 weeks' gestation prophylactic surfactant reduces neonatal mortality (RR 0.61, 95%CI 0.48–0.77; NNT 22, 95%CI 15–42) and pneumothorax (RR 0.62, 95%CI 0.42–0.89; NNT 48, 95%CI 27–200) compared to later treatment of established RDS [13]. This systematic review did not find a significant reduction in bronchopulmonary dysplasia (BPD) (RR 0.96, 95%CI 0.82–1.12) [13] but an earlier report using data from 3 randomized trials of poractant alfa found a reduction in chronic lung disease (CLD) in survivors at 28 days (adjusted OR 0.54, 95%CI 0.34–0.86) after adjustment for the following confounders: gender, birth weight, gestational age and prenatal administration of corticosteroids [16]. A subsequent analysis of these 3 trials of poractant alfa showed that prophylaxis significantly reduced severe intraventricular hemorrhage (IVH) compared to later treatment (OR 0.56, 95%CI 0.35–0.89) and this was especially apparent in preterm infants born outside tertiary perinatal centers (OR 0.11, 95%CI 0.02–0.49) [17]. All these trials of prophylaxis versus later treatment were conducted in the 1990s and could be criticized because of low rates of prenatal steroid use (about 20%) and relatively late treatment (1.5–6 hours after birth) in the comparison groups [1]. In a randomized trial of 651 infants of less than 28 weeks' gestation immediate (before the first breath) administration of calfactant was not superior to a post-ventilatory instillation of surfactant after initial resuscitation and stabilization at 10 minutes after birth [18].

Administration of surfactant generally requires endotracheal intubation and routine prophylaxis of infants less than 31 weeks' gestation might lead to unnecessary treatment in up to 50% of cases [1]. This will increase costs of care and could cause unwanted lung injury leading to BPD. On the other hand infants of 29–32 weeks' gestation treated with prophylactic calfactant showed clinical benefits compared to those treated at a median of only 1.5 hours later [19]. Nevertheless, currently because of increased use of prenatal steroids and early CPAP it is still not clear if prophylaxis is superior to early rescue treatment as soon as clinical signs of RDS develop [1].

70.3.3 Size of First Dose

Clinical trials used surfactant doses ranging from 25 to 200 mg phospholipids/kg [20]. These doses are all much larger by at

Table 70.1 Meta-analyses of prophylactic surfactant treatment of preterm infants

	Natural surfactant				Synthetic surfactant			
Outcome	RR	95%CI	NNT	95%CI	RR	95%CI	NNT/H	95%CI
Neonatal mortality	0.60	0.44 to 0.83	14	9 to 35	0.70	0.58 to 0.85	15	10 to 31
Pneumothorax	0.35	0.26 to 0.49	7	5 to 9	0.67	0.50 to 0.90	20	12 to 67
Pulmonary interstitial emphysema	0.46	0.35 to 0.60	6	4 to 8	0.68	0.50 to 0.93	16	9 to 77
Intraventricular hemorrhage	0.89	0.84 to 1.15	–	–	0.96	0.81 to 1.14	–	–
Severe IVH	1.22	0.90 to 1.66	–	–	1.01	0.75 to 1.38	–	–
Persistent ductus arteriosus	1.08	0.94 to 1.24	–	–	1.11	1.00 to 1.22	21	11 to 500
Retinopathy of prematurity	1.37	0.63 to 2.98	–	–	0.96	0.86 to 1.07	–	–
Severe ROP	0.58	0.27 to 1.24	–	–	0.89	0.58 to 1.36	–	–
Bronchopulmonary dysplasia	0.93	0.80 to 1.07	–	–	1.06	0.83 to 1.36	–	–

Data obtained from the Cochrane Library [3, 4]. *RR* relative risk, *CI* confidence interval, *NNT/H* number needed to treat or harm; *IVH* intraventricular hemorrhage, *ROP* retinopathy of prematurity, *BPD* bronchopulmonary dysplasia.

Table 70.2 Surfactant preparations in clinical use

Generic name	Trade name	Source	Phospholipids (mg/mL)	Dose (mg/kg)	Volume (mL/kg)	Manufacturer (country)
Beractant	Survanta	Bovine mince	25 (50% DPPC)	100	4	Abbott (USA)
BLES	bLES	Bovine lavage	27	135	5	BLES Biochemicals (Canada)
Bovactant	Alveofact	Bovine lavage	42	50	1.2	Thomae and Boehringer Ingelheim (Germany)
Calfactant	Infasurf	Bovine lavage	35 (74% DPPC)	105	3	ONY and Forest Labs (USA)
Poractant alfa	Curosurf	Porcine mince	80 (70% DPPC)	100 to 200	1.25 to 2.5	Chiesi Farmaceutici (Italy)
Surfactant TA	Surfacten	Bovine mince	30 (48% DPPC)	120	4	Tokyo Tanabe and Mitsubishi Pharma (Japan)

All preparations contain SP-B and SP-C in varying amounts. *BLES* bovine lipid extract surfactant.

least tenfold than the amount of lipids needed to form a monolayer on the alveolar surface of the lungs [11, 21]. For licensed surfactant preparations the recommended doses vary from 50–200 mg/kg with dose volumes of 1.2–5 mL/kg (Table 70.2) [1]. Larger doses of surfactant are superior to smaller ones [22, 23]. Surfactant TA in a dose of 120 mg/kg (100 mg phospholipids/kg) improved oxygenation and reduced BPD compared to 60 mg/kg [22]. When 100 mg/kg of bovactant was compared with 50 mg/kg the larger dose was associated with better improvement in oxygenation [23]. For poractant alfa 200 mg/kg gives a better acute response than 100 mg/kg [24, 25] and probably an improved survival [24]. The dose of surfactant required for optimal effects is not known but is probably at least 100 mg phospholipids/kg which is close to the 100-250 mg/kg estimated to form the total pulmonary surfactant pool in a full-term neonate [26]. It is possible that 100 mg/kg is sufficient for prophylactic treatment [2] but 200 mg/kg may give better outcomes for rescue therapy [25].

70.3.4 Method of Administration

Surfactants usually need to be administered directly into the lungs during at least a brief period of endotracheal intubation [1]. Preterm infants at high risk of developing RDS should be born in centers where personnel and equipment are readily available to allow appropriate care to begin from birth [6]. In some of the earlier clinical trials surfactant was administered as a bolus into each main bronchus or as a single bolus into the lower trachea whereas in other trials it was given as divided doses directed into each lung lobe by positioning the baby [21]. After instillation the baby is either manually ventilated for a short time or reconnected to the ventilator to distribute the surfactant. A sterile feeding tube is often used to deliver the surfactant through the endotracheal tube. Bolus administration has been compared with infusion over 30 minutes in an animal model with the former giving more uniform distribution [27]. However, a small clinical trial with beractant found no differences in outcome when 3 dosing procedures were compared [28] and this was supported by a study with poractant alfa, which compared a bolus dose with a 1 minute infusion through a dual lumen tube [29]. It is important to minimize duration of mechanical ventilation after surfactant administration as this is an independent risk factor for development of BPD [30]. The INSURE (INtubate SURfactant Extubate to CPAP) technique minimizes duration of ventilation and is useful for babies initially treated with CPAP who may avoid mechanical ventilation altogether [31]. Although more surfactant is used with INSURE the benefits of reduced air leak (RR 0.52, 95%CI 0.28–0.96) and BPD (RR 0.51, 95%CI 0.26–0.99) mean that this technique is recommended [32]. The benefits appear to be even greater when infants are treated at a lower threshold oxygen requirement of less than 45%.

Other methods to forgo need for endotracheal intubation include nebulization [33], direct tracheal instillation at laryngoscopy using a fine feeding catheter [34], intrapartum pharyngeal deposition [35] and use of a laryngeal mask [36] although none is presently in widespread use and all need to be further evaluated in large clinical trials [1].

70.3.5 Need for Redosing

At least 2 studies demonstrated that multiple doses (up to three) are superior to a single dose [37, 38] and this is confirmed in a systematic review [39]. Multiple dose treatment with poractant alfa reduced both neonatal mortality and pneumothorax in preterm infants with severe RDS [37]. This study used retreatment criteria based on continued need for mechanical ventilation and oxygen supplementation at 12 and 24 hours after the first dose and about two-thirds of babies needed retreatment. Currently babies with RDS are being treated with surfactant earlier or prophylactically and need for retreatment is much less than before. Poractant alfa in an initial dose of 200 mg/kg compared to 100 mg/kg lead to much less need for second and third doses [24, 25]. Although criteria for redosing in the past were fixed it is best to adopt a more flexible approach [1, 7]. Redosing of infants needing more than 30% oxygen has been compared with more than 40% in a randomized trial using calfactant [40]. Babies with uncomplicated RDS did equally well with the higher threshold retreatment criterion but about one-quarter of those enrolled

had complicated RDS (associated with birth asphyxia or sepsis) and they had a lower mortality when retreated earlier [40].

Specific recommendations by surfactant manufacturers differ slightly: for beractant retreatment may be given at intervals of at least 6 hours for up to 4 doses; for poractant alfa treatment may be repeated 12 hourly for 2 further doses if still intubated, and after prophylaxis may be repeated 6–12 hours later and after a further 12 hours [1]. However, the Canadian Paediatric Society recommends retreatment of infants needing more than 30% oxygen as early as 2 hours after the first dose [8] and the European Association of Perinatal Medicine recommends retreatment if there is ongoing RDS indicated by need for mechanical ventilation and supplemental oxygen [6]. The latter also recommends that babies extubated to CPAP should be retreated when they need more than 50% oxygen or if they are likely to need mechanical ventilation [6].

70.3.6 Type of Surfactant

Surfactant preparations studied in the 1990s were either synthetic (containing phospholipids without surfactant proteins) or natural (derived from animal lungs and containing both phospholipids and SP-B and SP-C) [1]. Of these the most well known synthetic surfactants were colfosceril palmitate and pumactant but they are no longer available for clinical use. The most well known natural surfactants are: beractant, calfactant, surfactant TA and bovactant (all of bovine origin) and poractant alfa (of porcine origin) (Table 70.2). Recently other surfactants have been produced in Cuba (Surfacen), Korea (Newfacten), Brazil and India but there is little information about them [1, 41]. Also, more recently studied are the so called "new generation synthetic surfactant preparations" [7] which contain phospholipids and surfactant peptide analogues such as lucinactant [42, 43] and recombinant SP-C surfactant [44] although the latter has not been studied in neonates.

As noted earlier studies comparing synthetic and natural surfactants provide evidence that the latter act more rapidly to improve pulmonary status and in the longer term they show improved survival with fewer pulmonary air leaks [12]. As a result the older synthetic surfactants, colfosceril palmitate and pumactant are no longer available as commercial products. There have been at least 10 trials comparing various natural surfactant preparations [1, 41] and the largest of these compared beractant with calfactant for both prevention and treatment of RDS [45]. Unfortunately, the planned sample size was not reached, although more than 2000 infants had been recruited to the 2 arms of the study, due to slow recruitment, but in those studied there was no trend to suggest different outcomes including survival without BPD [45]. Studies comparing beractant and poractant alfa are smaller but show that the latter produces a more rapid onset of action in preterm infants with RDS [25, 46]. A US trial of 293 preterm infants with moderately severe RDS compared 2 doses of poractant alfa (200 mg/kg and 100 mg/kg) with a 100 mg/kg dose of beractant and found that there was a reduced need for redosing with the higher dose of poractant alfa [25]. Furthermore, there was improved survival for infants of less than 32 weeks' gestation treated with the 200 mg/kg dose of poractant alfa compared with those treated with beractant. A meta-analysis of 5 randomized trials comparing poractant alfa with beractant confirmed the reduction in mortality with the former (RR 0.57, 95%CI 0.34–0.96; NNT 20, 95%CI 11–1000), although the benefit seemed to be limited to those infants treated with the higher dose of poractant alfa (RR 0.29, 95%CI 0.10–0.79; NNT 14, 95%CI 8–50) [46]. Despite the numbers of infants studied in these comparisons being small and the American Academy of Pediatrics stating that it is still "unclear whether significant differences in clinical outcomes exist among the available (animal-derived surfactant) products" [7], poractant alfa is now the most widely used surfactant preparation worldwide.

Two studies reported comparisons between lucinactant (a new synthetic surfactant) and colfosceril palmitate (with beractant as a reference group) [42] and poractant alfa [43] respectively. Lucinactant was more effective than colfosceril palmitate in reducing incidence of RDS at 24 hours and BPD at 36 weeks' postmenstrual age but was similarly effective as beractant [42]. Lucinactant was claimed to be similar in terms of efficacy and safety to poractant alfa [43] but this study was really too small to justify this [47] and there are concerns about early trial closure and limited statistical power [7]. A Cochrane review of these 2 lucinactant trials calls for further well designed studies of adequate size and power [48] and in the future this surfactant may be approved for use in neonates [1, 49]. Natural surfactants (beractant, calfactant and poractant alfa) remain the drugs of choice for prevention and treatment of RDS [1] and there is some evidence to support the superiority of poractant alfa [50].

70.3.7 Concomitant Interventions

Prenatal steroids, methods of respiratory support including CPAP and caffeine treatment will be considered in this section. There have been at least 21 randomized trials of prenatal steroids involving over 4000 infants at risk of RDS [51]. Treatment with prenatal steroids is associated with an overall reduction in neonatal mortality (RR 0.69, 95%CI 0.58–0.81), RDS (RR 0.66, 95%CI 0.59–0.73), intraventricular hemorrhage (RR 0.54, 95%CI 0.43–0.69), necrotizing enterocolitis (RR 0.46, 95%CI 0.29–0.74), respiratory support and intensive care admissions (RR 0.80, 95%CI 0.65–0.99) and systemic infections in the first 48 hours of life (RR 0.96, 95%CI 0.38–0.85) [51]. As prenatal steroids are also effective in women with premature rupture of membranes and pregnancy related hypertension they should be given in most cases where preterm birth is anticipated before 35 weeks' gestation. Furthermore, there is evidence of synergistic effects of prenatal steroids and postnatal surfactant [52] and therefore both are indicated in cases with high risk of RDS.

The combination of early surfactant treatment and CPAP was assessed in many studies [7, 32, 53–55] and one study compared nasal CPAP with intubation at birth in very preterm infants [56]. It is clear that the combination of CPAP and early surfactant administration reduces the need for mechanical ventilation particularly in infants of greater than 27 weeks' gestation [32, 53, 55] although the reduction in BPD is rather modest [32]. CPAP started in the delivery room without prophylactic surfactant in infants of 25–28 weeks' gestation does not significantly reduce the rate of death or BPD compared to intubation and is associated with a threefold increased risk of pneumothorax [56]. Until more information from trials [54] becomes available prophylactic surfactant followed by early CPAP if tolerated is recommended best practice for preterm infants of less than 27 weeks' gestation [2, 6]. To facilitate extubation after early surfactant administration by the INSURE technique administration of respiratory stimulants such as caffeine has been recommended [31].

There are other reasons for recommending caffeine administration for treatment of apnea of prematurity as this has been shown to reduce risks of BPD, persistent ductus arteriosus and need for ductus ligation [57]. The reduced incidence of BPD in infants less than 1250 grams may be due to shortening the duration of mechanical ventilation by about 1 week and at 18–20 months the surviving caffeine treated infants had better neurodevelopmental outcomes with less cerebral palsy [58]. There are clear benefits from treatment of very preterm infants with caffeine for apnea of prematurity and to facilitate extubation following surfactant therapy although further longer term follow-up studies are needed to confirm this. There is no clear evidence that early inhaled nitric oxide will prevent BPD in these very immature babies.

Management of persistent ductus arteriosus (PDA) in surfactant treated infants is discussed below with treatment of pulmonary hemorrhage. It is good practice to monitor surfactant treated infants for PDA and to use prostaglandin synthetase inhibitors (indomethacin or ibuprofen) early to prevent relapse of respiratory status and/or pulmonary hemorrhage.

70.3.8 Other Indications for Surfactant Therapy

Secondary surfactant dysfunction may occur in neonatal respiratory disorders other than RDS where the deficiency is primary [10]. Surfactant inactivation and secondary dysfunction probably occur in meconium aspiration syndrome, congenital pneumonia, pulmonary hemorrhage, acute lung injury or acute respiratory distress syndrome (ARDS) and early stages of BPD [7, 49]. There are varying degrees of evidence of effectiveness of surfactant replacement therapy in these conditions and the evidence base is much weaker than for RDS [1]. Four randomized trials of surfactant treatment in meconium aspiration syndrome are included in a systematic review that found improved oxygenation and a reduced need for extracorporeal membrane oxygenation (ECMO) in treated infants (RR 0.64, 95%CI 0.46–0.91; NNT 6) [1, 59]. However, there were no differences in risk of pneumothorax, CLD or mortality. These studies used a 6-hourly dosing regimen with up to 150 mg/kg of bovine surfactant for up to four doses and in general the improvement in oxygenation is not seen until after the third dose of surfactant [1, 59]. More recently dilute surfactant lavage has been used to try to remove meconium particles from the lungs and future studies may compare this technique with standard bolus dosing [60].

Surfactant inactivation is also present in neonatal pneumonia and a subgroup of term infants with this condition showed improved oxygenation and reduced need for ECMO in a small, randomized trial of beractant [61]. A larger observational study of poractant alfa in infants with group B streptococcal pneumonia showed similar short-term improvements in oxygenation but these were less impressive than those in preterm infants with RDS [62]. Although the number of infants with pneumonia and respiratory failure treated with surfactant is relatively small the improved oxygenation warrants further study and continued use of surfactant for this indication.

Pulmonary hemorrhage is now relatively uncommon but may still occur in very preterm infants following surfactant therapy and it has been postulated that this is due to rapidly improving pulmonary vascular resistance and large left-to-right shunt through a PDA [63]. It is sensible to monitor preterm infants after surfactant therapy for PDA clinically, echographically and by blood pressure assessment and to use either indomethacin or ibuprofen at an early stage to prevent pulmonary hemorrhage. Surfactant has been used to treat massive pulmonary hemorrhage and the rationale is that blood inhibits surfactant function. There is evidence of modest improvement in oxygenation after surfactant treatment but this comes from observational studies rather than randomized trials, which clearly would be difficult to perform [64].

In acute lung injury or ARDS surfactant is inactivated by proteins and other substances leaking into the alveolar spaces. Pneumonia and sepsis are frequently underlying causes and surfactant is at least partially effective in reversing signs of respiratory failure in these term infants [49, 61, 62]. In some preterm infants born to mothers with severe pre-eclampsia there may be delayed onset of respiratory distress which could be due to surfactant inactivation, a form of ARDS, and in these infants surfactant may be only partly effective with multiple doses being needed. Acute lung injury may be a prelude to development of BPD and surfactant has been used to treat infants with early CLD in a small observational study, which showed transient improvement in oxygenation [65]. Recently a bovine surfactant has been used as a vehicle to deliver budesonide to the airways of preterm infants with severe RDS with a view to preventing BPD [66]. In this relatively small randomized trial there was an unexpectedly large reduction in the combined outcome of death and BPD in infants treated with beractant plus budesonide compared to those treated with beractant alone

(32% versus 61%; p = 0.003) [66]. Clearly further larger trials of this approach to preventing BPD are needed.

Congenital diaphragmatic hernia is also associated with surfactant insufficiency but surfactant treatment in a large series of infants did not improve outcomes [7]. On the contrary there were increased rates of CLD, mortality and need for ECMO in surfactant treated infants [67]. Until more evidence is forthcoming surfactant replacement cannot be recommended for infants with congenital diaphragmatic hernia.

70.4 Future Developments

Further research is needed to more precisely define which infants benefit most from prophylactic surfactant as opposed to early treatment. Efforts should continue to reduce the rates of BPD while maximizing survival and this will mean limiting the duration of mechanical ventilation with judicious use of CPAP and perhaps caffeine. Alternative means of administering surfactant without the need for endotracheal intubation will be pursued and tested in large randomized trials. New generation synthetic surfactants are likely to eventually replace natural surfactants especially if they can be produced more cheaply and in greater quantities [1, 7]. There will also be further studies to widen the indications for surfactant treatment beyond prevention and treatment of RDS [1, 67]. In particular it is likely that surfactants will be used in future to deliver other drugs, such as budesonide [66] or other anti-inflammatory agents directly to the airways to ameliorate acute lung injury and prevent BPD.

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71

Nitric Oxide Therapy in Neonatology

John P. Kinsella

71.1 Background

After multiple approaches to the treatment of PPHN (see Chapter 67) since its first recognition as a disease marked by severe pulmonary hypertension, the discovery of the elusive "selective pulmonary vasodilator" markedly changed our understanding and clinical management of this syndrome. The most striking change in the management of PPHN in the last decade evolved from an improved understanding of the role of endogenous NO production and exogenous NO delivery on pulmonary vasoregulation.

Because the successful transition from fetal placental dependence to survival at birth requires that PVR rapidly declines and pulmonary blood flow increases, the role of NO in the transitional circulation has been intensively investigated to understand its relationship to the pathophysiology and treatment of PPHN.

NO was recognized as a potent vasodilator as early as 1979 [1], and in 1980, Furchgott and Zawadzki reported that acetylcholine-induced vasorelaxation was dependent on an intact endothelium through the elaboration of an endothelial-derived relaxing factor (EDRF) that diffused to the subjacent vascular smooth muscle [2].

In 1987 investigators from two separate laboratories reported that the biologic activity of EDRF was identical to NO or an NO-containing substance.

Palmer et al [3] induced the release of EDRF from porcine aortic endothelial cells in culture and compared the effects on superfused aortic strips with that of NO in solution. They found that the effects of EDRF were indistinguishable from those of NO. Ignarro et al [4] using a bioassay cascade superfusion technique with intrapulmonary arteries and veins, identified EDRF pharmacologically and chemically as NO, and found that EDRF and NO produced similar vasorelaxation and were inhibited by common antagonists.

Ignarro et al also recognized that NO was inactivated by combining with hemoproteins, and speculated that hemoglobin could trap endogenously produced NO that diffused into the vascular lumen, thus preventing any "downstream" vasorelaxation by this paracrine mediator.

The recognition that the endogenous production of this EDRF/NO mediator could be competitively blocked by modified L-arginine analogues prompted early experiments into the effects of NO in the fetal and transitional pulmonary circulation.

Abman et al performed the first experiments on the role of EDRF on the ovine fetal circulation, demonstrating that endogenous EDRF/NO production modulates basal pulmonary vascular tone in the late-gestation fetus, and that pharmacologic NO blockade inhibits endothelium-dependent pulmonary vasodilation [5]. These investigators also showed that pharmacologic NO blockade attenuates the rise in pulmonary blood flow at delivery, thus implicating endogenous NO formation in postnatal adaptation after birth and linking this laboratory observation to the life-threatening clinical condition of PPHN.

In addition, experiments using this ovine model showed that increased fetal oxygen tension augments endogenous NO release [6, 7], and the increase in pulmonary blood flow in response to rhythmic distention of the lung and high inspired oxygen concentrations are mediated in part by endogenous NO elaboration [8].

The observation that dilute NO gas could be therapeutically delivered by inhalation was first described by Higgenbottam et al who reported that brief (10 minute) inhalational NO treatment caused potent and selective pulmonary vasodilation in adults with severe pulmonary hypertension [9, 10]. Frostell et al demonstrated the selectivity of inhaled NO in an adult animal model of hypoxic pulmonary vasoconstriction [11], and the first description of the potent, sustained and selective vasodilator effect of inhaled NO in newborn lambs was reported by Kinsella et al in 1992 [12].

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

71.2 The Syndrome of Persistent Pulmonary Hypertension of the Newborn

The clinical syndrome of persistent pulmonary hypertension of the newborn (PPHN) occurs in association with various neonatal diseases, including meconium aspiration syndrome, group B streptococcal sepsis, and congenital diaphragmatic hernia, as well as from undetermined causes (idiopathic) (see Chapter 67). Although severe PPHN is commonly associated with near-term and term neonates, echocardiographic studies in premature infants with hyaline membrane disease (HMD) show that pulmonary hypertension may also complicate the course of severe HMD.

The treatment of PPHN commonly focuses only on pharmacologic therapy to reduce pulmonary vascular resistance. For the relatively small subset of neonatal patients with idiopathic PPHN who have pulmonary hypertension without concomitant lung disease, selective pulmonary vasodilation alone can cause marked improvements in oxygenation. However, PPHN often occurs with more common causes of neonatal respiratory distress, including disorders characterized by moderate to severe lung disease. Sepsis neonatorum and perinatal asphyxia can also severely compromise systemic vascular tone and cardiac performance, leading to systemic hypotension in addition to severe pulmonary hypertension. The pulmonary vasculature in many newborns with severe PPHN has marked structural changes, which include endothelial swelling, smooth muscle hypertrophy, and increased adventitial thickening. Moreover, functional abnormalities lead to altered responses to vasodilator and vasoconstrictor stimuli.

PPHN is a dynamic syndrome characterized by progressive changes in pulmonary vasoreactivity, cardiac performance, and parenchymal lung disease. The therapeutic approach to PPHN requires meticulous attention to all aspects of the cardiopulmonary perturbations (pulmonary hypertension, systemic vasodilation, decreased cardiac performance, and parenchymal lung disease) that characterize this syndrome and management is aided by serial hemodynamic, echocardiographic, and radiographic assessments (see Chapter 67).

71.3 Rationale for Inhaled NO Therapy

Early laboratory studies demonstrated that inhaled nitric oxide (NO) therapy caused marked and sustained reduction in pulmonary vascular resistance (PVR) in newborn animal models [12–14], and initial pilot studies showed marked improvement in oxygenation in term newborns with PPHN [15, 16]. Subsequent trials confirmed the safety and efficacy of inhaled NO in this population, and it is now an integral component of PPHN therapy [17–22].

As described above, the physiologic rationale for inhaled NO therapy in the treatment of PPHN is based on its ability to achieve potent and sustained pulmonary vasodilation without decreasing systemic vascular tone [23]. As a syndrome, PPHN is associated with diverse neonatal cardiac and pulmonary disorders that are characterized by high PVR causing extrapulmonary right-to-left shunting of blood across the arterial duct and/or oval foramen. The ability of inhaled NO therapy to selectively lower PVR and decrease extrapulmonary venoarterial admixture accounts for the acute improvement in oxygenation observed in newborns with PPHN [24]. However, oxygenation can also improve during inhaled NO therapy in critically ill patients who do not have extrapulmonary right-to-left shunting [25, 26]. Hypoxemia in these cases is primarily due to intrapulmonary shunting caused by continued perfusion of lung units that lack ventilation (e.g., atelectasis), with variable contributions form ventilation/perfusion (V/Q) inequality. Distinct from its ability to decrease extrapulmonary right-to-left shunting by reducing PVR, low dose inhaled NO therapy can also improve oxygenation by redirecting blood from poorly aerated or diseased lung regions to better aerated distal air spaces (microselective effect).

Finally, the diagnostic value of inhaled NO therapy is also important, in that failure to respond to inhaled NO raises important questions about the specific mechanism of hypoxemia. Poor responses to inhaled NO should lead to further diagnostic evaluation for unsuspected functional/structural cardiovascular or pulmonary disease.

71.4 Nitric Oxide Therapy in PPHN

Due to its selective pulmonary vasodilator effects, inhaled NO therapy is an important adjunct to available treatments for term newborns with hypoxemic respiratory failure. However, hypoxemic respiratory failure in the term newborn represents a heterogeneous group of disorders, and disease-specific responses have clearly been described. For example, patients with extapulmonary right-to–left shunting (PPHN) show acute improvement in oxygenation when PVR becomes subsystemic during NO therapy, and patients with predominantly intrapulmonary shunting (e.g., RDS) have less dramatic responses.

Several pathophysiologic disturbances contribute to hypoxemia in the newborn infant, including cardiac dysfunction, airway and pulmonary parenchymal abnormalities, and pulmonary vascular disorders. In some newborns with hypoxemic respiratory failure, a single mechanism predominates (e.g., extrapulmonary right-to-left shunting in idiopathic PPHN), but, more commonly, several of these mechanisms contribute to hypoxemia. For example, in a newborn with meconium aspiration syndrome, meconium may obstruct some airways decreasing V/Q ratios and increasing intrapulmonary shunting. Other lung segments may be over-ventilated relative to perfusion and cause increased physiologic dead space. Moreover, the same patient may have severe pulmonary hypertension with extrapulmonary right-to-left

shunting through the ductus arteriosus and foramen ovale. Not only does the overlap of these mechanisms complicate the clinical management, but the tendency for time-dependent changes in the relative contribution of each mechanism to hypoxemia requires continued vigilance as the disease progresses. Therefore, understanding the relative contribution of these different causes of hypoxemia becomes critically important as the inventory of therapeutic options expands.

Considering the important role of parenchymal lung disease in many cases of PPHN, pharmacologic pulmonary vasodilation alone may not be expected to cause sustained clinical improvement. The effects of inhaled NO may be suboptimal when lung volume is decreased in association with pulmonary parenchymal disease [27]. Atelectasis and air space disease (pneumonia, pulmonary edema) will decrease effective delivery of inhaled NO to its site of action in terminal lung units. In PPHN associated with heterogeneous (patchy) parenchymal lung disease, inhaled NO may be effective in optimizing ventilation-perfusion matching by preferentially causing vasodilation in lung units that are well ventilated. The effects of inhaled NO on ventilation-perfusion matching appear to be optimal at low doses (<20 ppm) [28, 29]. However, in cases complicated by homogeneous (diffuse) parenchymal lung disease and underinflation, pulmonary hypertension may be exacerbated because of the adverse mechanical effects of underinflation on pulmonary vascular resistance. In this setting, effective treatment of the underlying lung disease is essential (and sometimes sufficient) to cause resolution of the accompanying pulmonary hypertension.

Clinical trials of inhaled NO in the term newborn have incorporated ECMO treatment as an endpoint. Therefore, most patients have been enrolled in the first few days of life. Although one of the pivotal studies used to support the new drug application for inhaled NO therapy included as an entry criterion a postnatal age up to 14 days, the average age at enrollment in that study was 1.7 days [21]. Currently, clinical trials support the use of inhaled NO before treatment with ECMO, or usually within the first week of life. However, clinical experience suggests that inhaled NO may be of benefit as an adjuvant treatment after ECMO therapy in patients with sustained pulmonary hypertension (e.g., congenital diaphragmatic hernia). Thus, postnatal age alone should not define the duration of therapy in cases where prolonged treatment could be beneficial.

Studies support the use of inhaled NO in infants who have hypoxemic respiratory failure with evidence of PPHN, who require mechanical ventilation and high inspired oxygen concentrations. The most common criterion employed has been the oxygenation index. Although clinical trials commonly allowed for enrollment with OI levels > 25, the mean OI at study entry in multicenter trials approximated 40.

Thus it is unclear whether infants with less severe hypoxemia would benefit from inhaled NO therapy. However, Davidson et al reported a controlled clinical trial in which the average OI at study entry was 24±9 [30]. It is important to note that inhaled NO treatment did not reduce ECMO utilization in this study. Although entry criteria for this trial included echocardiographic evidence of pulmonary hypertension, only 9% of the patients had clinical evidence of right-to-left ductal shunting. Because of the mechanism of action of inhaled NO as a selective pulmonary vasodilator, it is likely that acute improvement in oxygenation caused by decreased pulmonary vascular resistance and reduced extrapulmonary right-to-left shunting would be most predictive of clinical improvement. Current multicenter studies suggest that treatment with inhaled NO may include an OI >25 with echocardiographic evidence of extrapulmonary right-to-left shunting.

The first studies of inhaled NO treatment in term newborns reported initial doses that ranged from 80 ppm to 6–20 ppm. Roberts et al reported that brief (30 minutes) inhalation of NO at 80 ppm improved oxygenation in patients with PPHN, but this response was sustained in only one patient after NO was discontinued [15]. In the second report, rapid improvement in oxygenation in neonates with severe PPHN was also demonstrated, but this was achieved at lower doses (20 ppm) for 4 hours [18]. This study also reported that decreasing the inhaled NO dose to 6 ppm for the duration of treatment provided sustained improvement in oxygenation. The relative effectiveness of low-dose inhaled NO in improving oxygenation in patients with severe PPHN was confirmed by a study by Finer et al [31]. Acute improvement in oxygenation during treatment was not different with doses of inhaled NO ranging from 5–80 ppm.

These laboratory and clinical studies established the boundaries of inhaled NO dosing protocols for subsequent randomized, clinical trials in newborns. Increasing the dose to 40 ppm does not generally improve oxygenation in patients who do not respond to the lower dose of 20 ppm. The initial dose in the NINOS trial was 20 ppm, but the dose was increased to 80 ppm if the improvement in PaO_2 was less than 20 torr [32]. In this study, only 3 of 53 infants (6%) who had little response to 20 ppm had an increase in PaO_2 >20 torr when treated with 80 ppm inhaled NO. Whether a progressive increase in PaO_2 would have occurred with continued exposure to 20 ppm could not be determined with this study design. Roberts et al initiated treatment with 80 ppm NO and subsequently weaned the inhaled NO concentration if oxygenation improved, thus the effects of lower initial inhaled NO doses could not be evaluated and the effects on ECMO utilization were not evaluated [18].

These studies did not systematically evaluate individual doses in an interpretable fashion. Davidson et al reported the results of a randomized, controlled, dose-response trial in term newborns with hypoxemic respiratory failure [30]. In this study, patients were randomized to treatment with either 0 (placebo), 5, 20 or 80 ppm NO. Each inhaled NO dose improved oxygenation compared to placebo, but there was no difference in responses between groups. However, at 80 ppm, methemoglobinemia (blood levels >7%) occurred in 13 of 37 patients (35%) and high inspired NO_2 concentrations (>3 ppm)

were reported in 7 of 37 patients (19%). Thus, 80 ppm inhaled NO was not more effective in improving oxygenation than 5 or 20 ppm, but was associated with adverse effects. Unfortunately, this trial was limited by early termination due to slow enrollment and the exclusion of lung recruitment approaches to optimize inhaled NO efficacy.

The available evidence, therefore, supports the use of doses of inhaled NO beginning at 20 ppm in term newborns with PPHN. Although brief exposures to higher doses (40–80 ppm) appear to be safe, sustained treatment with 80 ppm NO increases the risk of methemoglobinemia. The lowest effective starting dose for inhaled NO in term newborns with PPHN has not been determined. Cornfield et al reported that initiating treatment at 2 ppm does not acutely improve oxygenation and may diminish the subsequent response to 20 ppm [33]. However, this effect was not confirmed by Finer et al who found that initial exposure to low NO doses (1–2 ppm) did not compromise subsequent responses to higher doses (10–20 ppm), and dose increases were required in 80% of the low dose group [31]. Sustained improvement in oxygenation (after > 4 hours of treatment with 20 ppm) has been demonstrated for doses <10 ppm.

After improving oxygenation with inhaled NO therapy, strategies for weaning from NO become important. Numerous approaches have been employed, and few differences have been noted until final discontinuation of inhaled NO treatment. In one study, inhaled NO was reduced from 20 ppm–6 ppm after 4 hours of treatment without acute changes in oxygenation [30]. It is important to recognize that weaning inhaled NO is a different process than discontinuation of inhaled NO therapy.

In multicenter, clinical trials of inhaled NO therapy, the typical duration of inhaled NO treatment has been less than 5 days, which parallels the clinical resolution of PPHN. However, individual exceptions occur particularly in cases of pulmonary hypoplasia [34, 35]. If inhaled NO is required for longer than 5 days, investigations into other causes of pulmonary hypertension should be considered (e.g., alveolar capillary dysplasia), particularly if discontinuation of inhaled NO results in suprasytemic elevations of pulmonary artery pressure by echocardiography. In our practice, we discontinue inhaled NO if the FiO_2 is <0.60 and the PaO_2 is >60 mmHg without evidence of rebound pulmonary hypertension or an increase in FiO_2 > 15% after inhaled NO withdrawal.

Early clinical studies reported rapid and sometimes dramatic decreases in oxygenation and increases in PVR after abrupt withdrawal of inhaled NO during prolonged therapy. These responses are often mild and transient, and many patients with decreased oxygenation after inhaled NO withdrawal respond to brief elevations of FiO_2 and careful observation. In patients with a persistent need for treatment with higher inspired oxygen concentrations or with increased pulmonary hypertension after inhaled NO withdrawal, restarting inhaled NO treatment will generally cause rapid clinical improvement. In general, this so-called "rebound" response appears to decrease over time after more prolonged therapy. However, inhaled NO withdrawal can be associated with life-threatening elevations of pulmonary vascular resistance, profound desaturation, and systemic hypotension due to decreased cardiac output.

Mechanisms which contribute to these rebound effects are incompletely understood, but may be related to downregulation of endogenous NO production during exogenous NO therapy. Alternatively, the rise in pulmonary vascular resistance and drop in oxygenation after inhaled NO withdrawal may simply represent the presence of more severe underlying pulmonary vascular disease with loss of treatment effect of inhaled NO. The sudden increase in pulmonary artery pressure after rapid withdrawal of vasodilator therapy is not unique to inhaled NO, and has been observed in other clinical settings, such as prostacyclin withdrawal in adults with primary pulmonary hypertension and in postoperative cardiac patients.

Considering the important role of parenchymal lung disease in specific disorders included in the syndrome of PPHN, pharmacologic pulmonary vasodilation alone should be expected to cause sustained clinical improvement in many cases [36]. Moreover, patients not responding to inhaled NO can show marked improvement in oxygenation with adequate lung inflation alone. High success rates in early studies were achieved by withholding inhaled NO treatment until aggressive attempts were made to optimize ventilation and lung inflation with mechanical ventilation. These early studies demonstrated that the effects of inhaled NO may be suboptimal when lung volume is decreased in association with pulmonary parenchymal disease, for several reasons [37]. First, atelectasis and air space disease (pneumonia, pulmonary edema) may decrease the effective delivery of inhaled NO to its site of action in terminal lung units. Second, in cases complicated by severe lung disease and underinflation, pulmonary hypertension may be exacerbated because of the adverse mechanical effects of underinflation on pulmonary vascular resistance. Third, attention must be given to minimize overinflation to avoid inadvertent positive end expiratory pressure and gas trapping that may elevate pulmonary vascular resistance from vascular compression. This commonly complicates the management of infants with asymmetric lung disease or airways obstruction as observed in meconium aspiration syndrome.

In newborns with severe lung disease, HFOV is frequently used to optimize lung inflation and minimize lung injury [38]. In clinical pilot studies using inhaled NO, was found that the combination of HFOV and inhaled NO caused the greatest improvement in oxygenation in some newborns who had severe PPHN complicated by diffuse parencyhmal lung disease and underinflation (e.g., hyaline membrane disease, pneumonia) [39, 40]. Subsequently, a randomized, multicenter trial was conducted to determine the relative roles of inhaled NO and HFOV in newborns with severe PPHN [17]. In this study, treatment with HFOV+inhaled NO was more successful than HFOV or inhaled NO alone in severe PPHN, and differences in responses were related to the specific disease associated with the complex disorders of PPHN. For patients with PPHN

complicated by severe lung disease, response rates for HFOV+inhaled NO were better than HFOV alone or inhaled NO with conventional ventilation. In contrast, for patients without significant parenchymal lung disease, both inhaled NO and HFOV+inhaled NO were more effective than HFOV alone. This response to combined treatment with HFOV+inhaled NO likely reflects both improvement in intrapulmonary shunting in patients with severe lung disease and PPHN (using a strategy designed to recruit and sustain lung volume, rather than to hyperventilate) and augmented NO delivery to its site of action. In a single center randomized trial, Waffarn et al also reported improved responses to inhaled NO during HFOV when compared to conventional ventilation [41]. In this study, 13 of 14 infants randomized to HFOV and inhaled NO responded compared with 5 of 15 patients treated with conventional ventilation and inhaled NO. These findings underscore the importance of stratification of PPHN patients in NO trials, and the potential impact of adjuvant therapies on clinical responses to this potent and selective pulmonary vasodilator. Although inhaled NO may be an effective treatment for PPHN, it should be considered only as part of an overall clinical strategy that cautiously manages parenchymal lung disease, cardiac performance, and systemic hemodynamics.

71.5 Inhaled NO in the Premature Newborn

Early reports of iNO therapy in a premature newborn with pulmonary hypertension demonstrated marked improvement in oxygenation caused by effective treatment of severe pulmonary hypertension and resolution of extrapulmonary right-to-left shunting [42], as well as other preterm infants with severe respiratory failure [43, 44]. Subsequently, several randomized, controlled trials (RCTs) have confirmed the acute improvement in oxygenation caused by iNO treatment. However, in contrast to the direct pulmonary vasodilator effects of iNO, the focus of the most recently published studies has been on the potential beneficial effects of prolonged iNO administration on lung parenchymal and vascular development [45].

In a small, unmasked, randomized trial of iNO (20 ppm) and dexamethasone treatment, Subhedar et al reported no differences in survival, chronic lung disease, or intracranial hemorrhage between iNO treated infants and controls [46]. In a randomized, masked, multicenter clinical trial of low dose iNO therapy (5 ppm) in severely ill premature newborns with RDS who had marked hypoxemia despite surfactant therapy ($a/A\,O_2$ ratio < 0.10), iNO acutely improved PaO_2, but did not reduce the incidence of mortality or BPD [47]. Notably, there was no increase in the incidence or severity of ICH in this trial, and the incidence of the most severe ICH (grade 4) was 19% for the iNO group and 29% for the control group. The Franco-Belgian study group reported the results of an acute iNO response study (2 hour oxygenation endpoint); however, a brief duration of therapy and a high rate of crossover before the 2 hour trial endpoint compromised the interpretation of late outcome measures [48]. Hascoet et al reported the results of an unmasked, randomized trial of iNO in 145 premature newborns with hypoxemic respiratory failure [49]. They found no difference between the iNO and control groups in the primary outcome measure (intact survival at 28 days), and no differences in adverse events. As noted by Finer in an accompanying editorial, interpretation of the findings is limited by a relatively high rate of open-label iNO use and the lack of important outcomes such as death before discharge and BPD incidence at 36 weeks [50]. However, these investigators also studied the effect of low-dose iNO on serum markers of oxidative stress, and found that iNO treatment apparently reduced signs of oxidative stress in these patients [51]. Field et al described the findings of the UK INNOVO trial. In this unblinded study, 108 premature infants with severe hypoxemic respiratory failure were randomized to receive or not receive iNO [52]. There was no difference between the iNO and control group in the main outcome measures (death or severe disability at 1 year corrected age), and no difference in adverse events. Limitations of the study included an 8% crossover to iNO treatment, and treatment with other pulmonary vasodilators in 30% of the control group. Furthermore, Field et al described a lack of equipoise among investigators demonstrated by the observation that 75 infants eligible for enrollment were treated with iNO outside of the trial, leaving only infants with very severe lung disease enrolled in the study [53].

The largest trials of iNO therapy in premature newborns reported to date include the single center study of Schreiber et al [54], and the multicenter trials of Van Meurs et al [55], Ballard et al [56], and Kinsella et al [57]. All these studies were randomized, controlled and masked, but there were key differences in patient population, disease severity, dose and duration of therapy, and other factors.

Schreiber et al [54] randomized 207 infants to treatment with iNO or placebo. The main finding of the trial was a reduction in the incidence of BPD and death by 24% in the iNO group. These benefits appeared to accrue predominantly from a subset of newborns with relatively mild respiratory failure (OI<6.94). In addition to apparent pulmonary benefit caused by low-dose iNO, these authors also reported a 47% decrease in the incidence of severe ICH and periventricular leukomalacia (PVL). Furthermore, a subsequent report by the same group showed that the early decrease in ICH/PVL associated with iNO treatment manifested in improved neurodevelopmental outcome on follow-up examinations of this population [58]. In this follow-up study, 138 children (82% of survivors of the RCT) were evaluated for neurodevelopmental outcome at 2 years of age. In the group treated with iNO in the newborn period, 24% had abnormal outcomes (defined as cerebral palsy, blindness, hearing loss, or one score of less than 70 on the Bayley Scales of Infant Development II), in contrast to 46% in the control group.

Van Meurs et al [55] enrolled 420 newborns (401–1500 grams birthweight) in a multicenter RCT. Although the focus

of this study was on premature newborns and the major outcome measure was BPD, the design of the trial was similar to the previous NINOS trial in which term newborns were enrolled and acute changes in oxygenation determined continued treatment with study gas. That is, an acute dose-response study was performed and only patients who showed significant improvement in PaO_2 were continued on study gas. In contrast with other studies, the average duration of iNO treatment was only 76 hours. Overall, they found no difference in the incidence of death/BPD between the iNO and control groups. However, in post-hoc analyses, infants with birthweight >1000 grams showed a reduction in death/BPD following treatment with iNO (50% iNO versus 69% control). But a worrisome outcome was suggested in a post-hoc analysis of newborns weighing <1000 g. This analysis showed an increased risk of ICH/PVL (43% iNO versus 33% control). However, as noted in an editorial by Martin and Walsh [59], baseline ultrasound examinations were not performed, and it cannot be determined whether these very severely ill infants had ICH before iNO was initiated. Indeed, the severity of illness of infants in this trial of Van Meurs et al was also markedly different from the study of Schreiber et al. In the Van Meurs trial, the mean oxygenation index (OI) at enrollment for the iNO group was 23, compared to the median OI of 7.3 in the Schreiber study. This suggests that the degree of illness based upon the severity of respiratory failure may be related to iNO safety and efficacy in this population. However, an increased risk of ICH/PVL was not observed in a previous trial of iNO in premature newborns with severe hypoxemic respiratory failure (OI = 30) [47]. Other differences between these 2 trials may offer insights into the disparate outcomes, including the duration of iNO treatment (3 days versus 7 days), birthweight (839 g versus 992 g), and gestational age (26 weeks versus 27.4 weeks). Thus, Van Meurs et al enrolled smaller, more immature infants with severe respiratory failure who were treated relatively briefly with iNO, making direct comparisons between these 2 trials problematic.

The results of the two largest randomized, controlled and masked trials of iNO treatment in premature newborns were recently reported.

In the first trial, Ballard et al [56] randomized 582 premature newborns with birth weights of 500–1250 grams who required ventilatory support between 7 and 21 days of age. Infants were treated with study gas for a minimum of 24 days, and had an estimated OI of 7. They found that the incidence of survival without BPD was increased in the iNO treatment group (43.9%) compared to controls (36.8%, P = 0.042). A major finding of this trial was that the benefit of BPD reduction derived almost entirely from the subset of patients enrolled between 7–14 days, suggesting that early treatment is important to prevent BPD. There were no differences between the iNO and control groups in adverse events, including medical or surgical treatment of PDA. There were also no differences between the groups in ICH incidence, however infants were enrolled after the first week of life. Thus, this trial does not help inform the debate about iNO effects on brain injury in the premature newborn.

In the second trial [57], 793 premature newborns with birth weights of 500–1250 grams and requiring mechanical ventilation in the first 48 hours of life were randomized to treatment with 5 ppm iNO or placebo gas and treated for 21 days or until extubated. Overall, there was no difference in the incidence of death or BPD between groups. However, iNO therapy reduced the incidence of BPD for infants with birth weight >1000 g by 50% (p = 0.001). Low-dose iNO therapy reduced the incidence of PVL (p = 0.048), as well as the combined endpoints of ICH, PVL and ventriculomegaly for the entire study population (p = 0.032). INO therapy did not increase the incidence of adverse events, including mortality, ICH, PVL, pulmonary hemorrhage, and PDA treatment in any subgroup. In this trial there was no relationship between OI and brain injury risk, in contrast to the findings of Van Meurs et al. Mechanisms through which iNO therapy might provide neuroprotection in the premature newborn are uncertain, and warrant further study. Based on laboratory studies, several possibilities exist which include modulation of circulating cells (including neutrophils, monocytes and platelets) that may occur during NO exposure as they transit the pulmonary circulation. Alternatively, iNO induced down-regulation of lung derived cytokines may also reduce distant organ injury [60–62]. Another possible mechanism may relate to distal delivery of NO or NO-related metabolites through the systemic circulation through red blood cell or protein mediated pathways [63, 64].

The effects of iNO in the premature newborn may depend on the timing, dose, and duration of therapy, and the nature of the underlying disease. The available evidence from clinical trials suggests that low-dose iNO may be safe and effective in reducing the risk of death/BPD for a subset of premature newborns, in particular infants with birth weights > 1000 g. A neuroprotective effect of iNO has been demonstrated in large RCTs, but the relationship of disease severity and ICH/PVL risk is uncertain. Treatment of premature newborns with respiratory failure between 7–14 days after birth appears to be safe and effective in reducing the incidence of BPD.

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Extracorporeal Membrane Oxygenation for Neonates

Anne Greenough, Munir Agha and Adam P.R. Smith

72.1 Introduction

Extracorporeal membrane oxygenation (ECMO) is a form of cardiopulmonary bypass, modified such that patients can be supported for a prolonged period of time, even weeks. ECMO is not a treatment, but rather supports the patient until their lung and/or cardiac function improves. ECMO was developed for use in adult patients, but abandoned when initial trials failed to demonstrate that it improved survival advantage over that achieved by conventional respiratory support [1]. Subsequently, Bartlett and colleagues demonstrated the potential of ECMO for neonates with severe respiratory failure. Now, many thousands of neonates have been recorded on the Extracorporeal Life Support Organisation (ELSO) database as having received ECMO. In this chapter, the techniques used and the management of a neonate on ECMO are described. In addition, the indications for ECMO and the results achieved are discussed.

72.2 Techniques

There are two methods of providing ECMO; veno-arterial (VA) and veno-venous (VV). In VA ECMO, blood is drained from the right jugular vein and returned via the right carotid artery. The major advantage is that it provides support for both lung and heart function, as 80% cardiopulmonary bypass is achieved. Thus, the level of additional respiratory support can be reduced, decreasing the likelihood of further barotrauma. VA ECMO, however, has disadvantages, which include the potential for clots or air to enter the arterial circulation. In addition, the carotid artery is cannulated and arterial reconstruction may be difficult, particularly in small infants. In VV ECMO, the artificial lung is in series with the patient's lung and pulmonary and systemic perfusion relies on the patient's ventricular function. Venous blood is drained from the internal jugular vein and returned to the inferior vena cava via the femoral or, less commonly, the umbilical vein. Alternatively a double lumen catheter may be sited in the right atrium via the right jugular vein, venous blood is drained from one lumen into the ECMO circuit and oxygenated blood returned through the other cannula into the right atrium. VV ECMO obviates the need for carotid artery cannulation, but leg edema can occur following femoral vein ligation after decannulation.

72.3 ECMO Circuit

Venous blood is pumped through an oxygenator, which has blood and gas compartments separated by semi-permeable membranes, through which diffusion of oxygen and carbon dioxide occur. Oxygen transfer is dependent on the surface area of the membrane, pump flow and the degree of saturation of the venous blood. Carbon dioxide (CO_2) elimination is affected by the flow rate. Heat is lost, particularly due to the large surface are of the oxygenator, hence a heat exchanger is used to warm the blood prior to returning to the infant.

72.4 Management During ECMO

Heparin, usually as a continuous infusion (20–50 U/kg/hr) is given to maintain an activated clotting time of 180–200 seconds to prevent clotting in the circuit or cannulae. As a result of anticoagulation bleeding complications occur, most commonly at cannulation and surgical wounds. These risks can be minimized by maintaining adequate platelet counts (>100,000 cells/mm^3), a normal international normalized

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Table 72.1 Eligibility criteria for neonatal ECMO

Gestational age > 34 weeks
Birth weight > 2 kg
No lethal congenital anomalies
No irreversible brain injury
Mechanical ventilation < 14 days

ratio of prothrombin time (INR) and adequate levels of fibrinogen. Neuromuscular paralysis is necessary during cannula insertion, analgesia and sedation are continued throughout ECMO. Additional respiratory support is required during ECMO, but it is important to use settings that minimize further lung injury, but avoid atelectasis. Typical "lung rest" settings are peak pressures less than 30 cmH_2O, PEEP levels of 8–10 cmH_2O, rates of 10–25 breaths per minute and inspired oxygen concentrations of 0.21–0.40. Weaning is achieved by gradually reducing the pump flow rate.

72.5 Eligibility for ECMO

Prematurely born infants and those of low birth weight have an increased risk of intracranial hemorrhage and thus have been excluded from ECMO in most centers. ECMO, as it is a method of support rather than treatment, is inappropriate for infants with severe co-morbidities. Furthermore, the underlying pathology should have short-term reversibility, hence receiving more than 14 days of aggressive conventional ventilation is considered a relative contraindication to ECMO (Table 72.1). In the past, ECMO was generally reserved for infants with a predicted mortality of at least 80%; this has been defined as:

- oxygenation index (OI) > 40
- alveolar-arterial oxygen gradient ($AaDO_2$) ≥ 620 torr

$$OI = \frac{\text{mean airway pressure} \times FO_2}{PaO_2 \text{ (postductal)} \times 100}$$

$$AaDO_2 = FiO_2 \times (P_{atm} - 47) - PaO_2 - PaCO_2$$

where P_{atm} = atmospheric pressure (mmHg) and 47 mmHg reflects water vapor pressure.

In the UK ECMO trial [2] however, an OI >40 was associated with a mortality of only 41% on conventional ventilation.

72.6 Outcome

72.6.1 Mortality

The largest randomized neonatal ECMO trial recruited 185 neonates with severe respiratory failure from 55 neonatal intensive care units in the UK [2]. Parental consent to entry into the trial was requested if OI was greater than 40 or arterial partial pressure of CO_2 was >12kPa for three hours. Infants were then randomized to either remain on conventional therapy at their neonatal unit or be transferred for ECMO at one of five UK centers. Overall, there was a significant reduction in mortality for the ECMO group (relative risk (RR) 0.55, 95% CI 0.39–0.77; p = 0.0005). There was a benefit for ECMO in all diagnoses, although the effect in CDH infants was marginal; 17 CDH infants supported conventionally died before discharge and 14 of 18 supported with ECMO died before one year of age. Meta-analysis of the only four neonatal ECMO randomized trials demonstrated a benefit of ECMO on mortality (RR 0.44, 95% CI 0.31–0.61) [3].

Table 72.2 Survival rates to hospital discharge or transfer *

Primary Diagnosis	Total Cases	Survivors to discharge	% Survival
MAS	7,152	6,705	94
CDH	5,270	2,724	52
PPHN	3,452	2,681	78
Sepsis	2,506	1,884	75
RDS	1,437	1,209	84
Other	1,559	994	64

* Adapted from [10].

Up to July 2007, 21500 neonates supported for respiratory causes had been reported to the ELSO Registry [4]. The overall survival to hospital discharge or transfer was 76%; infants with MAS having the best results and those with CDH the poorest (Table 72.2). Approximately 10% of cases reported to the ELSO Registry have a primary cardiac problem, overall survival is 38%. ECMO may also be used during cardiopulmonary resuscitation.

72.6.2 Morbidity

Chronic respiratory, neurological and growth problems have been reported following ECMO. The only long-term data on outcome following randomization to ECMO or conventional management comes from follow-up of the UK ECMO trial. Follow-up at one year revealed that ECMO was associated with a reduction in mortality without an increase in severe disability (defined as having a Griffiths quotient less than 50 or being unable to participate in quantitative developmental assessment due to severity of disability). At four years of age, more of the ECMO surviving infants had no disability (50% versus 37%) [5]. Ninety of the infants (56 ECMO; 34 conventional) were seen at seven years, there were no significant differences in overall cognitive ability between the two groups; 76% of infants had overall performance within the normal range [6]. Overall, the study showed a continuing benefit of ECMO for the primary outcome of death or severe

disability (relative risk 0.64, 95% CI 0.47–0.86; p = 0.004). At one year of age, the ECMO-supported infants had better lung function [7]. At seven years of age, more children from the "conventional" group had evidence of respiratory morbidity, 32% had intermittent wheeze during the 12 months prior to questioning, and 41% regularly used inhalers, compared to 11% and 25% respectively of the ECMO group [6].

72.6.3 Cost-Effectiveness of ECMO

The costs of ECMO at one year are dominated by the expense of initial hospital care, partly reflecting the cost of ECMO provision itself, but also that the reduced mortality led to an increased average duration of stay in conventional neonatal intensive care. Cost-effectiveness improves over time, with the seven year follow-up analysis from the UK ECMO trial estimating the incremental cost per life year gained was £13,385 (2002–3 prices) [8]. Whilst treatment of infants was cost-effective in most diagnostic groups, the cost/ ratio for infants with CDH was far poorer.

72.7 Changes in ECMO Requirements with Time

Advances in other respiratory support techniques for term and near-term infants have reduced the number of cases referred for ECMO and altered the profile of these patients. Data from the ELSO registry highlight that neonatal respiratory cases fell from a peak of 1516 ECMO runs in 1992 to 751 runs in 2003 and that the proportion of cases with MAS fell from 35% to 25%, while CDH cases rose from 18% to 28% of neonatal respiratory cases. Similarly, a comparison of infants with severe respiratory failure treated at a single centre during two time periods showed a decrease in ECMO utilization from 42.8% to 27.7% [9]. There were concomitant increases in the use of HFOV (36.7% to 87.2%), surfactant (26.5% to 89.3%) and iNO (0% to 44.7%). One potential concern with the institution of newer methods of respiratory support is that it may delay ECMO treatment, which in non-randomized studies has been associated with prolongation of both ECMO and conventional treatment, as well as increased mortality [10].

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Lung Diseases: Problems of Steroid Treatment of Fetus and Newborn

Henry L. Halliday

73.1 Introduction

Corticosteroids given in the perinatal period have a multitude of biologic effects on the developing lung [1]. These include reduced alveolarization, increased production of surfactant lipids and proteins, increased absorption of lung fluid and increased antioxidant activity. Clearly some of these will be beneficial to the immature fetus or neonate whilst others will be detrimental. Thus administration of corticosteroids in the perinatal period must be based upon clinical judgment of the balance of benefits and risks.

The first true randomized trials of corticosteroids in the perinatal period were both published in 1972 in the same issue of a pediatric journal. They were aimed at either preventing or treating respiratory distress syndrome (RDS) a condition of predominantly preterm infants, which then had a high mortality rate. RDS is due to primary deficiency of pulmonary surfactant [2] and Liggins, an obstetrician in New Zealand had shown that an infusion of cortisol into fetal lambs seemed to prevent RDS [3]. He and a colleague from pediatrics performed a large randomized trial of prenatal betamethasone and showed that this not only decreased the risk of RDS in neonates but also reduced mortality. Since 1972 many randomized trials confirmed the benefits of prenatal corticosteroids [4] and long-term follow-up of the original cohort at age 31 years has been published [5]. Detrimental effects of prenatal corticosteroids and the controversy over any additional benefit of repeat courses will be discussed in this chapter.

The trial of Baden et al recruited only 44 infants with RDS to test the effects of hydrocortisone on blood gases, need for assisted ventilation and survival. Unfortunately, there were no beneficial effects of hydrocortisone and the authors also concluded that there were no immediate detrimental effects of the therapy. However, two subsequent follow-up publications raised concerns about an excess of severe intraventricular hemorrhage (IVH) and neurosensory and electroencephalographic abnormalities [6, 7] in hydrocortisone treated infants. Despite these early concerns dexamethasone was used to treat infants with bronchopulmonary dysplasia (BPD) and ventilator-dependence less than a decade later [8, 9]. The rationale for dexamethasone therapy was to reduce inflammation in the lung, an important precursor to the development of BPD. However, the doses of dexamethasone used in these trials were very large (about 0.5 mg/kg/day as a starting dose) giving pharmacologic rather than physiologic effects. It was not surprising that the acute beneficial effects on lung function [8, 9] would later be shown to have had adverse long-term effects on the developing central nervous system [10].

73.2 Results from Randomized Trials of Prenatal Corticosteroids

The most recent systematic review of a single course of prenatal steroids for accelerating fetal lung maturation includes 21 studies and 4269 infants [4]. The authors conclude that prenatal steroid treatment does not increase risk to the mother of death, chorioamnionitis or puerperal sepsis. However, treatment was associated with an overall reduction in neonatal death (RR 0.69; 95%CI 0.58–0.81), RDS (RR 0.66; 95%CI 0.59–0.73), IVH (RR 0.54; 95%CI 0.43–0.69), necrotizing enterocolitis (NEC) (RR 0.46; 95%CI 0.29–0.74), respiratory support and intensive care admissions (RR 0.80; 95%CI 0.99) and systemic infections in the first 48 hours of life (RR 0.56; 95%CI 0.38–0.85). Prenatal steroid use is also effective in women with premature rupture of membranes and pregnancy related hypertension syndromes [4]. The authors concluded that a single course of prenatal steroids should be considered routinely for preterm delivery with few exceptions. Further information is needed concerning optimal dose to delivery interval, optimal steroid to use, effects in multiple pregnancies and to confirm the long-term effects into adulthood [4]. The most recent systematic review of repeat doses of prenatal

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steroids for women at risk of preterm birth for preventing RDS included 5 trials and involved over 2000 women [11]. Treatment with repeat dose(s) of steroids was associated with a reduction in both occurrence (RR 0.82; 95%CI 0.72–0.93) and severity of any neonatal lung disease (RR 0.60; 95%CI 0.48–0.75) and serious infant morbidity (RR 0.79; 95%CI 0.67–0.93). Mean birth weight was not significantly different between treatment groups, although in one trial, treatment with repeat dose(s) of steroids was associated with a reduction in birth weight Z score and in two trials with an increased risk of being small for gestational age (SGA) at birth (RR 1.63; 95%CI 1.12–2.37). The authors concluded that repeat dose(s) of prenatal steroids reduce the occurrence and severity of neonatal lung disease and the risk of serious health problems in the first few weeks of life. The short-term benefits for babies support the use of repeat dose(s) of prenatal steroids for women at risk of preterm birth. However, these benefits are associated with a reduction in some measures of weight and head circumference at birth, and there is still insufficient evidence on the longer-term benefits and risks [11].

Since this repeat dose systematic review was published another important large study reported that multiple courses of prenatal steroids, every 14 days, do not improve preterm birth outcomes and are associated with decreased weight, length and head circumference at birth [12]. These authors concluded that a repeat treatment schedule of every 14 days until week 33 weeks or delivery was not recommended. Recently, a balanced view has emerged stating that weekly repeat doses of prenatal steroids compared with a single course seem to reduce neonatal respiratory morbidity and some of its complications, particularly when delivery occurs at less than 32 weeks' gestation [13]. The major concern about repeat doses of steroids is that they may be associated with adverse effects on the brain and that long-term outcome data are currently lacking. A compromise may be to consider repeated doses of steroids for women who remain at very high risk of extreme preterm delivery (for example < 29 weeks), where a 20% reduction in composite severe morbidity may outweigh the theoretical concerns of risks in later childhood [13].

73.3 Results from Randomized Trials of Postnatal Steroids

The most recent systematic reviews of postnatal steroids for preventing [14] and treating chronic lung disease (CLD) [15] in preterm infants were published in the Cochrane Library in 2009. Twenty-eight trials enrolling 3740 infants were included in the systematic review of postnatal steroids given in the first week of life to try to prevent CLD in preterm infants [14]. There were significant benefits of early postnatal steroids as regards earlier extubation and decreased risks of CLD at both 28 days (RR 0.87; 95%CI 0.81–0.93) and 36 weeks' postmenstrual age (PMA) (RR 0.79; 95%CI 0.71–0.88). There were also decreases in persistent ductus arteriosus (PDA) (RR 0.78; 95%CI 0.72–0.85), retinopathy of prematurity (ROP) (RR 0.88; 95%CI 0.80–0.97) and severe ROP (RR 0.79; 95%CI 0.65–0.97). There were no significant differences in rates of mortality, infection, severe IVH, periventricular leukomalacia, NEC or pulmonary hemorrhage. Important adverse effects included gastrointestinal bleeding (RR 1.86; 95%CI 1.35–2.55), intestinal perforation (RR 1.81; 95%CI 1.33–2.48), hyperglycemia (RR 1.34; 95%CI 1.21–1.48), hypertension (RR 1.85; 95%CI 1.55–2.22), hypertrophic cardiomyopathy (RR 4.33; 95%CI 1.40–13.4) and growth failure (RR 6.67; 95%CI 2.27–19.6). Twelve trials reported late outcomes and several adverse effects were found including developmental delay (RR 1.94; 95%CI 1.30–2.88), cerebral palsy (RR 1.50; 95%CI 1.13–3.59) and abnormal neurological examination (RR 1.81; 95%CI 1.33–2.47). However, the rates of the combined outcomes of death or cerebral palsy, and death or major neurosensory disability were not significantly increased [14].

Dexamethasone was used in 20 studies and hydrocortisone in eight. In a subgroup analysis most of the beneficial and harmful effects were attributable to dexamethasone although with hydrocortisone there was an increase in intestinal perforation (RR 2.02; 95%CI 1.13–3.59) and a borderline reduction in PDA (RR 0.85; 95%CI 0.73–0.99) [14]. The authors concluded that the benefits of early postnatal steroid treatment (< 8 days), particularly with dexamethasone, may not outweigh the known or potential adverse effects of this treatment. There is a compelling need for the long-term follow-up and reporting of late outcomes, especially neurological and developmental outcomes, among surviving infants who participated in all randomized trials of early postnatal steroids. Hydrocortisone in the doses and regimens used in these trials had few beneficial or harmful effects and cannot be recommended for prevention of CLD [14]. One randomized trial of a 4-week course of early dexamethasone reported outcomes at school age [16]. The authors' conclusion was that early postnatal dexamethasone therapy should not be recommended for the routine prevention or treatment of CLD because it leads to substantial adverse effects on neuromotor and cognitive function at school age [16].

Nineteen trials enrolling a total of 1345 infants were included in the systematic review of late (after 7 days) postnatal steroids for CLD in preterm infants [15]. Late steroid treatment was associated with a reduction in neonatal mortality (RR 0.49; 95%CI 0.28–0.85) but not at discharge (RR 0.87; 95%CI 0.65–1.17). Other beneficial effects included earlier extubation and reductions in CLD at both 28 days (RR 0.87; 95%CI 0.81–0.94) and 36 weeks' PMA (RR 0.72; 95%CI 0.61–0.85), need for late rescue treatment with dexamethasone (RR 0.46; 95%CI 0.36–0.58), discharge home on oxygen therapy (RR 0.71; 95%CI 0.54–0.94) and death or CLD at both 28 days (RR 0.84; 95%CI 0.78–0.89) and 36 weeks' PMA (RR 0.72; 95%CI 0.63–0.82). There was a trend towards an increased risk of infection and gastrointestinal bleeding but not NEC. Short-term adverse effects included hyperglycemia (RR 1.52; 95%CI 1.25–1.84), glycosuria (RR 8.03; 95%CI 2.43–26.5) and hypertension (RR 2.66; 95%CI 1.58–4.49). There was an increase

in severe ROP (RR 1.38; 95%CI 1.07–1.79) but no significant increase in blindness. There was a trend towards reduction in severe IVH (RR 0.44; 95%CI 0.19–1.02) and trends towards an increase in cerebral palsy and abnormal neurological examination were partly offset by a trend in the opposite direction in death before late follow-up. The combined rate of death or cerebral palsy was not significantly different between steroid and control groups. Major neurosensory disability and the combined rate of death or major neurosensory disability were not significantly different between groups. There were no substantial differences between groups for other outcomes in later childhood, including respiratory health or function, blood pressure or growth [15]. The authors concluded that the benefits of late steroid therapy (after 7 days) may not outweigh actual or potential adverse effects. Although there continues to be concern about an increase in adverse neurological outcomes in infants treated with early postnatal steroids, this review of late steroids suggests that this may reduce neonatal mortality without significantly increasing the risk of adverse long-term neurodevelopmental outcomes. However, the methodological quality of the studies determining long-term outcomes is limited in some cases and no study was sufficiently powered to detect increased rates of important long-term outcomes. Given the evidence of both benefits and harms of treatment and the limitations of the evidence at present it appears prudent to reserve use of late postnatal steroids to infants who cannot be weaned from mechanical ventilation and to minimize the dose and duration of any course of treatment [15].

73.4 Conclusions

There are some problems with steroid treatment of the fetus and newborn but on the whole if used according to accepted guidelines the benefits outweigh the risks. For the fetus at risk of preterm birth a single course of betamethasone leads to lung maturity and reduces the risk of RDS, neonatal mortality and other important complications of prematurity [5]. The current recommendation is to give 2 doses of 12 mg betamethasone, 24 hours apart, to women who may deliver within 7 days and are less than 35 weeks pregnant [17].

There remains some controversy about repeat courses of prenatal steroids as they are associated with reduced fetal growth but consensus seems to support their use when there is a high risk of preterm birth before 29 weeks' gestation [13].

The beneficial pulmonary effects of early postnatal dexamethasone, in the first week of life, do not outweigh the adverse effects on neurodevelopment [14]. However, late postnatal steroid therapy, after the first week of life, seems to be associated with similar beneficial pulmonary effects without significant increase in neurodevelopmental sequelae [15]. Late steroid therapy, with low dose, short duration dexamethasone is probably still indicated for preterm infants with CLD who remain ventilator dependent and have severe respiratory disease [15, 18].

The role of inhaled steroids needs further evaluation in randomized clinical trials [19].

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Apnea of Prematurity and Sudden Infant Death Syndrome

Christian F. Poets

74.1 Apnea of Prematurity

74.1.1 Introduction

Apnea of prematurity (AOP) is a developmental and thus self-resolving disorder, which nonetheless can cause serious problems. Almost every infant born at less than 29 weeks gestation exhibits AOP. No study, however, has yet identified a threshold in either frequency or severity of accompanying bradycardia or hypoxemia above or below which there is an increased risk for neurodevelopmental impairment. Most even suggested that within the frequency and severity of AOP tolerated in their institution, there is little indication that AOP, by itself, leads to impaired neurodevelopment [1].

Given the lack of good outcome data, indications for treatment or a step-up in its intensity are bound to be arbitrary. From a physiological point of view it is not the apnea but its effect on oxygenation and/or heart rate that is relevant to the well-being of an infant. Although never systematically investigated, it is unlikely that a desaturation to 79% for 2 s will pose the same risk on neurodevelopment as one to 20% requiring vigorous stimulation. In the author's institution, an apnea severity score has therefore been developed to encompass this situation (Fig. 74.1).

This score however, is as yet unvalidated and can only serve as an example of how apnea severity may be assessed more objectively even without documentation via continuous recordings. It also has to be kept in mind that any treatment threshold strongly depends on the averaging time used by the instrument with which SpO_2 and heart rate are monitored (Fig. 74.2).

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74.1.2 Treatment

Interventions to improve AOP can be grouped according to 3 underlying pathophysiologic mechanisms: interventions aimed at (i) reducing work of breathing, (ii) increasing respiratory drive, and (iii) improving diaphragmatic contractility (Table 74.1).

74.1.2.1 Interventions Aimed at Reducing Work of Breathing

Prone Head-Elevated Positioning

In the prone position, the chest wall is stabilized and thoracoabdominal asynchrony reduced. Several studies have demonstrated that the prone position reduces apnea rate in preterm infants, with some also reporting a decrease in desaturation rate. An extension of the prone position is the prone, head-elevated tilt position, which was associated with a 49% reduction in desaturations to <85% in one study. Recently, however, 2 studies re-investigated the issue, triggered by the observation that infants appear more comfortable when only the chest rather than the entire body is being tilted. Both found a slight (–13%) reduction in the frequency of desaturation/bradycardia compared to the horizontal position; one even found no advantage for the head-up tilt position [2]. This much less clear advantage of the head-up tilt postion may be due to the fact that infants in the earlier study had received no other treatment, whereas in the more recent ones, all had received caffeine and most continuous positive airway pressure (CPAP) [2].

Thus, the effect of head-up positioning on bradycardia and intermittent hypoxia may be less pronounced in infants already receiving other treatments for AOP. The prone, head-up tilt position should therefore be considered as a first-line intervention for infants with AOP, but will likely offer little additional effect in infants already treated with caffeine or CPAP.

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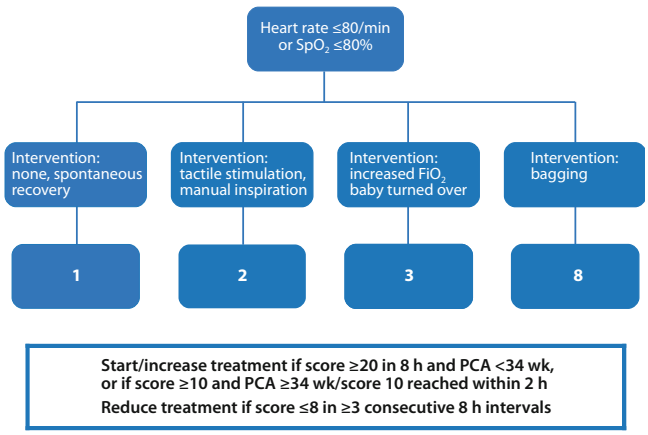

Fig. 74.1 Apnea Score to assess apnea severity similarly across different raters/infants. The value in the lower row of boxes indicates the respective score

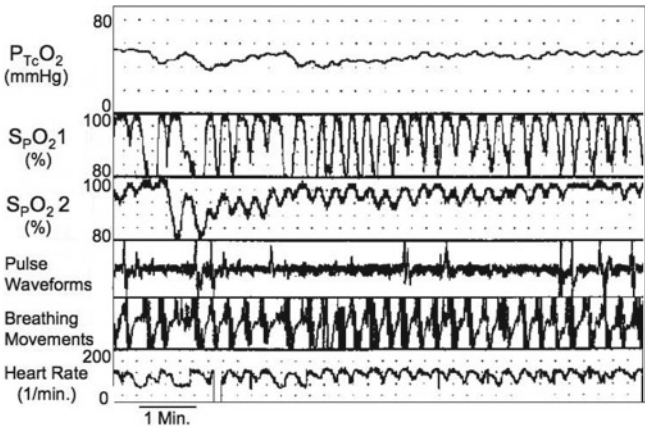

Fig. 74.2 Influence of averaging time of the pulse oximeter on rates of intermittent hypoxia. Two pulse oximeters (Nellcor N200) are attached to the right and left foot of a preterm infant during periodic apnea. The upper pulse oximeter saturation trace (SpO_2 1) was recorded in a beat-to-beat mode, the lower one (SpO_2 2) in a 6-s averaging mode. Note that with the oximeter set in a non-averaging mode, 15 alarms to ≤80% SpO_2 would have been counted, in contrast to only two with the 6-s averaging mode. Unaccounted for, this purely technical difference might have resulted in different treatment decisions. *$PTcO_2$*, partial pressure of transcutaneous oxygen tension, *SPO_2* pulse oximeter saturation. From [1], with permission

Table 74.1 Suggested incremental treatment plan for AOP

1st Step:	Prone, 15° head-up tilt position
2nd Step:	Caffeine*
3rd Step:	Variable flow CPAP or synchronized N-IPPV
4th Step:	Intubation and mechanical ventilation**

*Consider caffeine as first-line treatment in infants <28 weeks GA. **Doxapram may be considered as an alternative in infants who continue to exhibit recurrent hypoxia and bradycardia despite mechanical ventilation.

Continuous Positive Airway Pressure and Nasal Ventilation

CPAP has been shown to reduce extubation failure in preterm infants, despite the fact that most systems currently available do not reduce work of breathing. CPAP can be applied via a nasopharyngel tube or (bi-)nasal prongs. Reintubation rates are 40% lower with the latter (relativ risk (RR) 0.59 [0.41;0.85], number needed to treat 5 [NNT]) [3], which is why this should be the preferred mode when applying CPAP. An extension of CPAP is nasal intermittent positive pressure ventilation (N-IPPV), which has a high effectiveness over CPAP in preventing extubation failure (RR 0.21 [0.10–0.45], NNT 3) [4].

Typically, an inspiratory pressure of 15–20 cm H_2O, applied at a rate of 10–20/min, is combined with a CPAP level of 5–6 cm H_2O. There is theoretical concern that this might result in gastric distension, but this has not been confirmed [4]. Both studies showing superiority of N-IPPV over CPAP in the meta-analysis, however, used synchronized nasal ventilation [4]. When we compared non-synchronized N-IPPV with N-CPAP delivered via a variable flow device that reduces work of breathing, the rate of bradycardia and desaturation was 50% lower with the latter device [5]. Thus, a reduced work of breathing may be key to success for nasal ventilatory support to improve AOP, which can be achieved via either synchronized N-IPPV or variable flow N-CPAP devices [5].

74.1.2.2 Interventions Aimed at Increasing Respiratory Drive

Oxygen Administration

That oxygen stabilizes neonatal respiration was first observed in 1923. Several crossover trials in infants with and without BPD have since shown that the application of low-flow oxygen results in a reduced rate of intermittent hypoxia and apnea [6]. Application of this therapy, however, has to be weighed against side effects potentially resulting from oxygen toxicity. Thus, before the data from randomized controlled trials (RCT) currently underway are available, no ideal oxygen level can be recommended.

Increased Inspiratory CO_2 Concentration

Respiratory drive also depends on CO_2. If CO_2 levels fall to below the eupneic baseline value, apnea occurs. Recently, an inspiratory CO_2 concentration of 0.8% for 2 h was as effective as theophylline in reducing apnea duration and rate [7]. It is likely, however, that infants will quickly accommodate to a higher inspiratory CO_2 concentration; thus, data on the longer-term effectiveness of this treatment modality are required.

Red Blood Cell Transfusions

An increase in respiratory drive resulting from an increased tissue oxygenation is also one of the proposed mechanisms for red cell transfusions to potentially ameliorate AOP, and anemia has indeed been implicated in the pathophysiology of AOP. It would thus seem logical to hypothesize that blood transfusions are an effective treatment modality in infants with AOP who are anemic. Data on the effect of blood transfusions on the frequency of these episodes, however, are conflicting. In 2 crossover-studies, focussing on bradycardia and intermittent hypoxia, we found no effect of transfusion [8, 9]. Recently, however, Bell et al published the results of a RCT comparing a liberal with a more restrictive transfusion policy in 100 VLBW infants [10]. Mean rate of apneas requiring stimulation was 0.23/h in the liberal *vs* 0.46/h in the restricted transfusion group, but significantly more infants in the restrictive transfusion group happened to have brain lesions and may thus have been more likely to develop AOP. Based on these conflicting data, blood transfusion to anemic VLBW infants with AOP as their only symptom cannot be recommended.

Caffeine

Methylxanthines increase chemoreceptor sensitivity as well as respiratory drive and can also improve diaphragmatic contractility. Of the substances available, caffeine has a wider therapeutic range and fewer side effects than theophylline. There were concerns, however, that caffeine, being an adenosine antagonist, could reduce tolerance to hypoxia and thus might be harmful to infants with recurrent hypoxia. Thus, it is most commendable that Schmidt et al performed a large placebo-controlled RCT enrolling over 2000 infants [11]. Caffeine (or placebo) was started during the first 10 days of life in infants of 500–1250 g birth weight at a dose of 5–10 mg/kg caffeine citrate until no longer needed for AOP treatment. Mechanical ventilation, CPAP, and oxygen could all be discontinued approximately one week earlier in infants treated with caffeine. Somewhat unexpectedly, and not a primary endpoint, was the finding of a 40% lower risk of bronchopulmonary dysplasia (BPD; 36% *vs* 47%; OR 0.6; 95% CI 0.5;0.8) and a 30% lower risk of developing a symptomatic patent ductus arteriosus (OR 0.7; [0.5;0.8]) in the caffeine group [11]. Most important, however, are the data on the primary outcome, i.e., death or disability at 18 month corrected age. These showed that caffeine was associated with a 23% reduction in this outcome (OR 0.77; 95% CI 0.64–0.93). This benefit was particularly strong for cerebral palsy: 4.4% *vs* 7.3% of infants had this outcome (RR 0.58; 0.39–0.87) [12].

In a subgroup analysis, the effect of caffeine on the primary outcome was found to be restricted to those requiring ventilatory support at randomization; i.e., caffeine had no effect on death or disability in infants not requiring CPAP or IPPV. Interestingly, the reduced duration of the need for ventilatory support was only evident in those who were randomized within the first 3 days of life. Thus, caffeine appears to be the only drug yet identified to reduce cerebral palsy. Given that this effect appears to be restricted to those receiving ventilatory support, it is most likely that it was mediated via a reduced need for mechanical ventilation rather than a reduced rate of bradycardia and desaturation, but this has yet to be proven. In any case, caffeine administration should be started within the first 3 days of age in infants <1250 g requiring respiratory support who suffer from or are likely to develop AOP.

Doxapram

Doxapram stimulates peripheral chemoreceptors at low and central ones at high doses. It shows a dose-response curve. Most studies used a continuous intravenous (i.v.) infusion, although some suggest that the i.v. solution may also be given orally at twice the dose. Short-term side effects become quite common at doses above 1.5 mg/kg/h and include irritability, myoclonus, elevated blood pressure and gastric residuals. Of concern is the fact that the long-term effects of doxapram are unknown. This is particularly worrying given that in a study on factors associated with poor motor development in extremely low birthweight infants, the only difference found was that infants with developmental delay had received a mean cumulative doxapram dose of 2233 mg, compared to 615 mg in controls ($p<0.01$) [13]. Although such a retrospective analysis cannot distinguish whether this reflects sequelae of severe AOP (for which doxapram had been given), or a direct drug effect, it clearly raises concern. In the caffeine study [12], infants in the placebo group had not only been more likely to develop cerebral palsy, but were also 3 times more likely to receive doxapram. Given these data (or lack thereof), doxapram cannot be recommended as a standard treatment for AOP.

74.1.2.3 Interventions Aimed at Improving Diaphragmatic Strength

If muscle fatigue is involved in AOP, then improvements in diaphragmatic strength should be beneficial, but this has hardly been tested yet. Fifteen years ago, an enriched total parenteral nutrition (TPN) solution with branched-chain amino acids, which improve diaphragmatic function *in vitro*, was associated with a decrease in the average number of episodes of apnea from 58 during standard TPN to 11 with the enriched solution infusion during matched 12-hour periods ($p < 0.01$) in an unblinded cross-over study design [14]. This interesting approach to the treatment of AOP should be investigated further.

In summary, treatment for AOP may follow an incremental approach, starting with infant care procedures such as prone

positioning, followed by methylxanthines and CPAP/N-IPPV. We urgently need data on how much intermittent hypoxia/bradycardia can be tolerated in an individual infant without putting her/him at risk of developmental impairment.

74.2 Sudden Infant Death Syndrome (SIDS)

Sudden deaths in infants have been known since biblical times, but it was only in 1970 that Bergman et al coined the term SIDS. It was recently re-defined by an expert panel as "the sudden unexpected death of an infant <1 year of age, with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including performance of a complete autopsy and review of the circumstances of death and the clinical history". To facilitate research, this definition was stratified into cases with the "classic" features of SIDS present and completely documented (so-called Category 1A SIDS), those with these features present, but incompletely documented (IB), and those meeting Category I criteria except for one or more of the following: Age <3 wk or >270 d, similar deaths in siblings, close relatives or infants in the custody of the same caregiver, perinatal conditions such as a history of premature birth, suspected mechanical suffocation, or marked inflammatory changes not sufficient to be assigned a cause of death (Category II) [15]. According to this definition, all cases not meeting criteria for category I or II SIDS, including those for which an autopsy has not been performed, are now called "Unclassified Sudden Infant Death" [15]. The practicality of this new definitory approach remains to be seen.

74.2.1 Epidemiology

74.2.1.1 Incidence

Despite a recent decline in incidence, SIDS continues to be the leading cause of postperinatal mortality in developed countries (Table 74.2). There is now a trend to classify many deaths previously diagnosed as SIDS into other categories such as (positional) asphyxia. In fact, a recent analysis of US mortality data showed that concurrent increases in post-neonatal mortality rates for unknown and unspecified causes and suffocation accounted for 90% of the decrease in SIDS rates in 1999 to 2001 [16]. Thus, it is important always to report changes in both, SIDS and post-neonatal mortality rates (to which SIDS contributes substantially).

Table 74.2 SIDS and post-neonatal mortality (PNM) rates in different countries, 1990 *vs* 2003 [27]

Country	PNM 1990	PNM 2003	Change (%)	SIDS 1990	SIDS 2003	Change (%)
Netherlands	2.3	1.2	–48	0.56	0.10	–82
Sweden	2.4	0.96	–60	1.0	0.23	–77
Germany	3.3	1.4	–58	1.42	0.43	–70
USA	3.38	2.67	–31	1.30	0.54	–58
New Zealand	4.21	1.90	–55	2.90	0.80	–72

74.2.1.2 Age and Time of Death

One of the most striking epidemiological features in SIDS is its characteristic age distribution. Some 75% of deaths occur between 2–4 months of age, and 95% before 9 months of age [1]. Early beliefs that SIDS is extremely rare in the neonatal period cannot be maintained: 6–7% of SIDS victims are younger than 1 month of age, and 11% of all neonatal deaths are due to SIDS [1]. There are even several reports of sudden death in apparantly healthy neonates occurring while sleeping on their mother's abdomen shortly after delivery.

Throughout the year, there used to be a preponderance of deaths in the cold season, but this has almost disappeared following the back-to-sleep campaigns. In a study from the UK, only 27% of deaths occurred between December and February [17].

74.2.1.3 Risk Factors

A large number of factors associated with an increased risk of SIDS have been identified (Table 74.3) [1]. Many underline the importance of social factors in the pathogenesis of SIDS; others, like maternal smoking or anemia during pregnancy, suggest that there must already be a disturbance during intrauterine life that poses a risk to the infant. Factors that are potentially amenable to modification include maternal

Table 74.3 Risk factors for SIDS. Derived from [1], with permission

Maternal factors	Infantile factors
Young age	Male gender
Multiparity	Low birth weight
Smoking during pregnancy	Low birth length
Maternal drug abuse	Premature birth
Previous fetal deaths	Blood type B
Anemia during pregnancy	Low Apgar scores
Placenta praevia	Low hematocrit at 48 hours
Premature rupture of membranes	Not using a pacifier
Low social class	Prone or side sleeping position
Low family income	Bed-sharing
Short inter-pregnancy interval	Overheating
Unmarried mother	Not breastfed
Partner unemployed	Siblings in family
Late attendance of antenatal clinic	Sleeping in own room
Postnatal depression	Previous SIDS in family
Attendance to psychiatrist	Previous cyanotic episode
Urinary tract infection in pregnancy	

smoking, not breast feeding, not using a pacifier, overheating, and a non-supine sleeping position, and these are the factors targeted by most intervention campaigns. Recently, however, "new" modifiable risk factors have been identified. These include head covering, which has a pooled adjusted OR of 16.9 (95% CI 13.6–22.7) and a population-attributable risk (PAR) of 27% [18], and prone sleeping in preterm and low birth weight infants [19]. Parents of the latter infants may get confused by the common use of the prone position in hospital; it is thus particularly important to place these infants on their back before hospital discharge and to explain the importance of supine sleeping to their parents. Bed-sharing is also a relatively "new" risk factor, at least in infants <11–13 wk, where it is associated with a 10- to 19-fold increase in SIDS risk [20, 21]. It should thus be strongly discouraged in the first 3 months of life. Thereafter, no increased risk has been found.

74.2.2 Pathology

74.2.2.1 Intrathoracic Petechial Hemorrhages

As implied by its definition, there is no morphological finding in SIDS that sufficiently explains death. There are, however, a number of characteristic findings in these infants, such as serosal petechiae, which are so consistent that they appear to support the concept that SID may indeed form a specific disease entity. Other characteristic findings in SIDS include the occurrence of (often bloody) froth around the nose and mouth. Both may result from high transpulmonary pressure swings, such as occurring during breathing against an obstructed upper airway [1].

74.2.2.2 Abnormalities in Brainstem Serotonergic System

Serotonin is a neurotransmitter involved in regulating various processes potentially related to SIDS, e.g., sleep and arousal, control of breathing, airway reflexes, and autonomic function [22]. Endogenously released serotonin is also required for gasping. Comparative studies on the binding properties of neurotransmitter receptors in SIDS found multiple brainstem abnormalities in SIDS compared to control infants, suggesting that such abnormalities may be involved in some SIDS cases [22].

74.2.2.3 Genetic Studies in SIDS

For many years, it has been known that some infants who die suddenly and unexpectedly do so because they have an inherited disease such as the A984G mutation in the medium-chain acyl-CoA dehydrogenase (MCAD) gene. More recently, mutations in genes encoding cardiac ion channels have been identified. These well-defined diseases, however, contribute only a few percent to all cases of SIDS (and, by definition, prohibit defining SIDS as the cause of death). More relevant to SIDS are recent data on gene polymorphisms that may predispose infants to SIDS under certain circumstances, such as those found in the serotonin transporter gene. The reader is referred to a recent review on this exciting new field of research [23].

74.2.3 Pathophysiology

There are now a number of recordings from cardiorespiratory monitors that were obtained during SIDS. Despite some limitations, these recordings have for the first time provided us with objective data on the pathophysiological mechanisms immediately preceding SIDS. In an analysis of 9 recordings of chest wall impedance and heart rate from infants who had an autopsy diagnosis of SIDS or mild BPD, gasping was the predominant pattern, being already present at the time of the monitor alarm in 3 infants and occurring within 3 min after it in a further 4 (Fig. 74.3) [24]. Primary trigger for the monitor alarm had been bradycardia in all but two infants, but there was no indication of heart block or ventricular tachycardia. These observations, confirmed by a similar, more recent study [25], suggest that prolonged apnea is unlikely to be a primary mechanism in the sequence of events leading to most cases of SIDS, while the "primary" bradycardia is most likely caused by hypoxemia. In addition, there seems to be some failure or depression of arousal and gasping. The

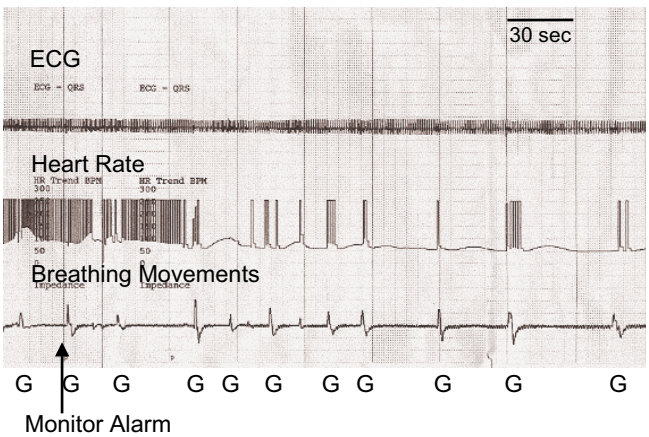

Fig. 74.3 Section from a memory monitor printout of a 1-month old infant born at 34 wk gestation and with an autopsy diagnosis of SIDS. There are 11 gasps, which progressively decrease in amplitude. There is an increase in heart rate from 72 to 140 bpm during the first 20 s of recording, followed by several smaller increases in heart rate that appear to occur in response to the gasps and are also decreasing in amplitude over time. *G*, gasp. From [24], with permission

underlying causes of both the hypoxemia and the failure of these infants to resuscitate themselves from this hypoxemia remain to be determined.

74.2.4 Prevention

74.2.4.1 Primary Prevention

Dissemination of advice on safe sleeping has been one of the most effective health interventions ever performed. Recommendations are reviewed at regular intervals to incorporate new data. The current advice given by the American Academy of Pediatrics includes the following (abbreviated) [26]:

- infants should be placed for sleep in a supine position for every sleep;
- use a firm sleep surface. pillows or sheepskins should not be placed under a sleeping infant;
- keep soft objects and loose bedding out of the crib;
- let your baby share your room for sleep, but not your bed;
- do not smoke during pregnancy, and keep him/her smoke-free after birth;
- consider offering a pacifier at nap time and bed time;
- avoid overheating. Bedroom temperature should be comfortable for a lightly clothed adult.

74.2.4.2 Secondary Prevention

For many years, the use of home monitors in specific risk groups, i.e., secondary prevention, has been the only method to prevent SIDS, but its effectiveness in reducing its incidence has never been proven. Nonetheless, home monitoring may be prescribed as a diagnostic tool or as an early warning of potentially dangerous pathophysiology. The first group comprises infants after an apparent life-threatening event (ALTE) and infants from families who had 2 or more sudden unexpected infant deaths. The second group involves technology-dependent infants (e.g., with a tracheostomy), infants with respiratory control disorders and preterm infants with persistent AOP. AOP is not associated with an increased risk of SIDS, but monitoring may be indicated to prevent potential sequelae, e.g., cerebral palsy. Whatever the indication, a pulse oximeter with motion-resistant technology to reduce the frequency of false alarms is currently the preferred monitoring device. Although there are no controlled trials on this issue, there is evidence to suggest that apnea and hypoxemia may occur too late during the events leading to death always to allow for successful resuscitation. Monitoring should continue for 4 weeks after the last (true) monitor alarm except for siblings from families with multiple deaths who should be monitored until the age of the oldest infant who died.

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75

Cardiovascular Physiology, Pathology, and Clinical Investigation

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75.1 Introduction

Congenital heart defects (CHD) are a heterogeneous entity, representing the most common malformations in the fetal and neonatal period. The incidence of moderate to severe structural CHD that will require expert cardiologic care is 2.5 to 3 per 1000 live births. Moderately severe forms of CHD probably account for another 3 per 1000 live births. Specialised cardiologic care is not needed for most minor forms of CHD, such as tiny ventricular septal defect (VSD) or atrial septal defect (ASD) [1]. Most infants with congenital heart disease are identified by the end of neonatal period.

75.2 The Fetal and Neonatal Circulation

Fetal circulation is shown in Fig. 75.1.

In the fetal circulation, the right and left ventricles work in a parallel circuit. Three cardiovascular structures, unique to the fetus, are important for maintaining this circulation: ductus venosus, foramen ovale and ductus arteriosus. The umbilical vein carries oxygenated blood (PO_2 of about 30–35 mmHg) from the placenta to the inferior vena cava (IVC) through the ductus venosus, where it partially mixes with poorly oxygenated IVC blood derived from the lower part of the fetal body. When it reaches the right atrium (RA), oxygenated blood preferentially streams across the foramen ovale (FO) and enters the left atrium (LA), where it mixes with the pulmonary venous return before entering the left ventricle (LV). From the LV, the ascending aorta supplies fully oxygenated blood (PO_2 about 28 mmHg) to the coronary arteries, the head, and the upper extremities. Only a small portion of the LV cardiac output streams through the aortic arch and supplies flow to the thoracic aorta. Superior caval vein blood, which is considerably less oxygenated (PO_2 12–14 mmHg), enters the RA where it is directed preferentially across the tricuspid valve into the right ventricle (RV). Just about 10% of RV output is ejected into the pulmonary arteries (PA) because the fetal lungs are still not expanded

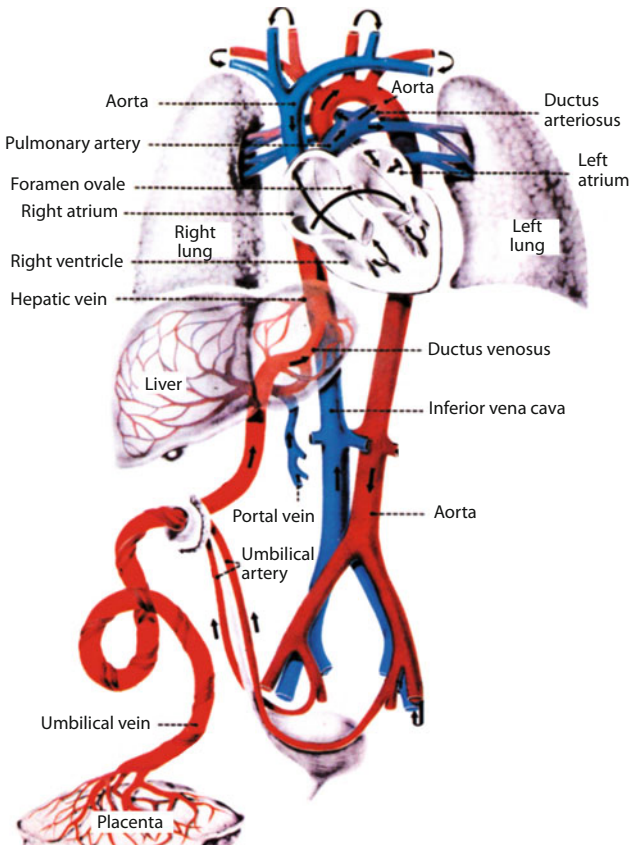

Fig. 75.1 Diagram of fetal circulation showing the four sites of shunt: placenta, ductus venosus, formanen ovale and ductus arterious (modified with permission from Fesslova V. Ecocardiografia fetale. Raffaello Cortina Editore, Milano 2008, p. 39)

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

and ventilated, and pulmonary vascular resistances is therefore very high. Most RV output bypasses the lungs and flows through the ductus arteriosus into the descending aorta and the inferior part of the fetal body, after which it returns to the placenta via the two umbilical arteries.

A distinctive feature of fetal circulation is the role of the placenta, low-resistance vascular bed, where oxygenation and metabolites exchange occur. The placenta is not only an organ for a gas exchange, and the fetus adapts by producing fetal hemoglobin which has a higher affinity for oxygen, compared to adult hemoglobin.

The dimensions of fetal cardiac chambers reflect the difference between right and left sided blood volume, with the RV and pulmonary artery being larger respectively than the LV and ascending aorta, although the pressure in both circuits is equal due to the patency of the ductus arteriosus.

75.3 Changes in Circulation after Birth

At birth, the main changes in circulation occur as follows: first the placental circulation disappears and the pulmonary circulation is established due to (i) a rapid increase in systemic vascular resistance (SVR) as a result of very-low-resistance placenta circulation removal and (ii) closure of the ductus venosus. Second, physical expansion of the lungs results in: (i) reduction of the pulmonary vascular resistance (PVR), fall in PA pressure and increment of pulmonary blood flow; (ii) mechanical and passive closure of the FO due to a difference between left and right atrial pressure; pressure in the left exceeds that in the right because of increased pulmonary venous return to the LA and decreased venous return to the RA after ductus venosus closure; (iii) closure of patent ductus arteriosus (PDA) secondary to increased arterial oxygen saturation.

75.4 Regulation of Pulmonary Vascular Resistance and Pulmonary Blood Flow

In fetal life the PVR is high and the oxygen concentration, at the level of smooth muscle cells in the pulmonary arteriolar walls, is low. At birth, since lung ventilation occurs, PVR promptly decreases and simultaneously pulmonary blood flow increases. Those events are associated with thinning of the medial layer of the pulmonary arterioles as well as hyperplasia of alveolar units and their own circulatory vessels. After the initial sudden decrease in PVR and pressure, there is a slow progressive decrease within 2–6 weeks. Several factors may interfere with the pulmonary flow or pulmonary vascular resistance, causing conditions such as persistent pulmonary hypertension or delay in PVR normalization process:

- hypoxia and/or altitude;
- acidosis;
- severe hyaline membrane disease or other pulmonary diseases;
- congenital heart defects such as large VSD or PDA, which are characterized by high PA pressure, secondary to direct transmission of the systemic pressure to the PA through the large defect;
- increased pressure in the LA or pulmonary veins.

75.5 Closure of the Ductus Arteriosus

During fetal life, the ductus arteriosus is fully patent and pulmonary and aortic pressures are therefore equal. Once the duct closes, pulmonary artery pressure decreases as the resistance in the pulmonary vascular bed falls. The functional closure of the PDA occurs because of constriction of the medial, smooth muscle cells within 10 to 15 hours after birth. The following factors are relevant to ductal closure:

- *Oxygen* A postnatal increase in systemic circulation oxygen saturation is the strongest stimulus for smooth muscle cell constriction.
- *Gestational age of the newborn* The ductus arteriosus is more likely to remain open in preterm infants because of immaturity of the constrictor response to oxygen by ductal smooth muscle cells.
- *Prostaglandin E (PGE)* An acute decrease in PGE levels results in ductal constriction. This event is determined by placenta removal, since the placenta is a significant source of PGE. Drugs like indomethacin, a prostaglandin synthetase inhibitor, can be used to effect closure of a significant PDA in premature infants.
- *Hypoxia and acidosis* relax the duct but constrict pulmonary arterioles which are also constricted by sympathetic stimulation and α-adrenergic stimulation.
- *Oxygen* constricts the duct but relaxes pulmonary arterioles.

75.6 Persistence of the Fetal Circulation

Persistent pulmonary hypertension of the newborn (PPHN) or persistence of fetal circulation occurs in approximately 1 in 1500 live births. This neonatal condition is due to failure of pulmonary vascular transition to extrauterine life. This clinical syndrome is characterized by persistence of pulmonary hypertension which causes varying degrees of cyanosis depending on the amount of right-to-left blood flow (shunting) through the PDA or patent foramen ovale (PFO). No underlying congenital heart defect is present. In this situation, PVR exceeds the systemic vascular resistance; in postnatal life it causes reduced pulmonary perfusion and systemic hypoxemia. There is often some degree of myocardial dysfunction with elevated pulmonary vascular resistance characterized by decreased contractility or tricuspid regurgitation. These abnormalities are caused by global or subendocardial ischemia and are aggravated by hypoglycemia and hypocalcemia.

The most common precipitating disease is meconium-aspiration syndrome, sepsis/pneumonia, perinatal acidosis, abnormal pulmonary vascular development, or pulmonary hypoplasia.

The differential diagnosis includes: congenital heart disease, sepsis, pulmonary parenchymal disease, polycythemia, intrauterine or perinatal asphyxia and metabolic disease, myocardial dysfunction due to intrauterine constriction of the ductus arteriosus.

Diagnostic evaluation of the cyanotic newborn infant includes the gradient in oxygenation, chest X-ray and echocardiography. The aim is to rule out CHD. The only structural abnormality is the presence of a large PDA with right-to-left or bidirectional shunt. The RV is enlarged, with flattening of the interventricular septum. The RA is also enlarged with the atrial septum bulging towards the left with or without an interatrial communication. Left ventricular systolic and diastolic dysfunction may also be present.

Initial treatment includes an immediate attempt to reverse hypoxemia, improving pulmonary and systemic perfusion. Respiratory support, correction of any metabolic disorder and hemodynamic support should be provided.

75.7 Clinical Presentation

In spite of a wide spectrum of CHD, clinical presentation does not vary much. Signs and symptoms of severe heart disease in the newborn period include cyanosis, discrepancy of the arterial pulses and/or four limb blood pressure, and congestive heart failure. Circulatory collapse is uncommon but the emergency treatment of shock and clinical stabilization should precede definitive anatomic diagnosis.

75.7.1 Cyanosis

Cyanosis is a blue discoloration of the skin caused by deoxygenated blood within the capillary network. Detection of cyanosis depends on the skill and experience of the physician and environmental light (it is more evident in natural than in artificial light). Cyanosis does not always indicate hypoxemia: a neonate may be severely hypoxemic but not clinically cyanotic. Cyanosis can be detected on many parts of the body: lips, fingernails, toenails, tip of the nose, oral mucous membranes, conjunctivae and tip of the tongue.

By definition cyanosis is determined by deoxygenated hemoglobin (Hb) concentration exceeding 3 g/dL in arterial blood and/or 5 g/dL in capillary bed.

Fetal and adult hemoglobin are different in terms of oxygen affinity, which is indicated by the P50 value. P50 is the level of PaO_2 (partial pressure of oxygen) at which 50% of hemoglobin is bound to O_2. It is about 27 mmHg in adults. The P50 of fetal hemoglobin is about 20 mmHg, the venous oxygen saturation (O_2 sat) is therefore higher in the neonate (about 80%) than in the adult (about 70% or less). As a consequence, cyanosis in neonates becomes clinically evident at lower level of PaO_2 than in adults or children, about 38–39 mmHg and 52–54 mmHg respectively.

The level of hemoglobin greatly influences the appearance of cyanosis. In patients with polycythemia, cyanosis is detectable at a higher level of oxygen saturation; conversely in patients with anemia, cyanosis appears at lower level of oxygen saturation. In polycythemic neonate (Hb 22 g/dL) cyanosis becomes evident when O_2 sat approaches 85–92%. In a normal neonate the usual level of Hb is about 17 g/dL and cyanosis becomes evident when O_2 sat approaches 82% and PaO_2 38–39 mmHg. Often preterm babies have a reduced Hb concentration, about 12 g/dL or less, in which case cyanosis appears at O_2 sat level of about 75%.

Cyanosis may be either peripheral or central. Peripheral cyanosis is a bluish discoloration limited to the extremities, associated with normal arterial O_2 saturation and pink color of mucous membranes; it can be seen with acrocyanosis or exposure to cold. The blue color may reflect intermittent vasomotor changes in the skin. A pathological cause of acrocyanosis is cutaneous arterial constriction resulting from low cardiac output state with sluggish peripheral blood flow. Central cyanosis is cyanosis associated with arterial oxygen desaturation.

Generally cyanosis related to CHD is not associated with respiratory distress, unlike cyanosis due to lung disease. Exceptions to this rule are:

- Central cyanosis with low PaO_2 in the 20s or less, in which cases hyperpnea is stimulated.
- Cyanosis associated with low cardiac output. This is commonly seen with left heart obstructive lesions if the ductus arteriosus becomes restrictive. The pulse volume will be poor and the extremities cool.
- Cyanosis associated with obstruction of pulmonary venous drainage resulting in pulmonary edema, such as is seen in obstructed total anomalous pulmonary venous return.

75.7.1.1 Differential Cyanosis

Differential cyanosis in neonates occurs when cyanosis is more evident in the lower extremities than the upper or viceversa.

- Upper limb saturation higher than the lower limb: deoxygenated blood coming from the RV shunts from pulmonary artery to distal aorta through the ductus arteriosus because of either elevated PVR or aortic obstruction proximal to the ductus such as in critical aortic coarctation or interrupted aortic arch.
- Lower limb saturation higher than upper limb: typical of this condition is a reversed flow into the ascending aorta and aortic arch from pulmonary artery through a large ductus arteriosus, such as in transposition of great arteries, large PDA and pulmonary hypertension.

Differential cyanosis is fairly uncommon and is of little practical diagnostic use.

75.7.1.2 Differential Diagnosis

Cyanosis is one of the most common clinical presentations of diverse disease processes, including cardiac, lung and central nervous system abnormalities. Table 75.1 summarizes the differential diagnosis of cyanosis.

A suggested plan for investigation of the cyanotic newborn baby includes:

1. Clinical evaluation of cyanosis: central or peripheral, extension, localization. Heart rate, arterial pulses, blood pressure, respiratory rate, presence of respiratory distress.
2. Chest X-ray: may reveal a pulmonary or cardiac cause, and the severity of the problem.
3. Arterial blood gas analysis in room air: confirms or excludes central cyanosis.
4. Hyperoxia test: helps in differentiating pulmonary or central nervous system causes from cardiac causes.
5. ECG if a cardiac cause of cyanosis is suspected.
6. Umbilical arterial line: PaO_2 in a preductal artery (e.g. a radial artery) 10 to 15 mmHg higher than the PaO_2 in a postductal artery (e.g. from an umbilical arterial line) suggests a right-to-left ductal shunt.
7. Prostaglandin E_1: to be commenced if a cyanotic defect is suspected as a result of investigations.

Table 75.2 summarizes the differential diagnoses of CHF in the neonate.

Table 75.1 Differential diagnosis of cyanosis

Newborns with CHD	
CHD with increased pulmonary vascularity	*CHD with decreased pulmonary vascularity*
– D-Transposition of great arteries	– Tetralogy of Fallot
– Total anomalous pulmonary venous return	– Double outlet right ventricle with PS
– Taussig-Bing anomaly	– Ebstein's anomaly
– PPHN	– Pulmonary atresia
– Truncus arteriosus	– Tricuspid atresia
– Single ventricle without PS	– Single ventricle with PS
– TGA and VSD	– TGA and PS
Pulmonary disease	
– Pneumothorax or pleural effusion	
– Diaphragmatic hernia	
– Persistent fetal circulation syndrome	
– Perinatal asphyxia	
Central nervous system	
Cyanosis with normal PaO_2	

PS pulmonary stenosis, *PPHN* persistent pulmonary hypertension of the newborn, *TGA* transposition of the great arteries, *VSD* ventricular septal defect.

75.7.2 Heart Failure in the Newborn

Congestive heart failure (CHF) as clinical presentation accounts for about 30% of infants and children with CHD [2]. CHF is a clinical syndrome in which cardiac output is insufficient to meet the metabolic demands of the tissues, including oxygen and nutrient delivery and waste removal.

In early stages infants may be tachypneic, tachycardic with increased respiratory effort, and there may be hepatomegaly and delayed capillary refill. In worsening CHF dyspnea with grunting, intercostal and subcostal recession may appear. Feeding difficulties, failure to thrive and diaphoresis may occur later.

An acute presentation as cardiorespiratory collapse may occour with "left sided" lesions.

75.7.2.1 Differential Diagnosis

When heart failure develops in the first weeks of life, the differential diagnosis includes:

– structural heart disease causing significant volume and/or pressure overload;
– myocardial disease;
– arrhythmia.

Table 75.2 Causes of heart failure in newborns

Structural Heart Disease	
– Anomalies of coronary circulation – Ebstein's anomaly of tricuspid valve – Hypoplasic left heart syndrome	*At birth*
– Transposition of the great arteries – Premature infants with large patent ductus arteriosus – Total anomalous pulmonary venous return	*First week*
– Critical aortic or pulmonary stenosis – Coarctation of the aorta – Structural cardiac disease with left-to right shunt at level of ventricles – Tetralogy of Fallot or Pulmonary atresia with aorto-pulmonary collaterals	*1-4 weeks*
Myocardial Disease	
A *Primary* – Myocarditis – Transient myocardial ischemia (with or without birth asphyxia) – Cardiomyopathy (as seen in infants of diabetic mothers)	
B *Secondary* – Sustained tachyarrhythmias (atrial flutter or fibrillation) – Congenital heart block – Birth asphyxia resulting in transient myocardial ischemia – Severe anemia (as seen in hydrops fetalis) – Overtransfusion or fluid overload – Metabolic causes: hypoglycemia, hypocalcaemia – Neonatal sepsis	

75.7.3 Heart Murmur

To determine the sensitivity and specificity of clinical assessment of murmurs in neonates, as performed by a pediatric cardiologist, and to identify clinical features that predict the presence of CHD, Mackie et al [3] reported that clinical assessment detected most complex CHD whereas some simple lesions were missed. The murmur quality, location, and timing were predictive of CHD. The prevalence of the heart murmur in premature infants is higher than in full-term infants.

There are three common innocent murmurs in the newborn period:

1. A pulmonary flow murmur of the newborn is the most common. It is best heared at the upper left sternal border and transmits well to both sides of the chest. The murmur is soft, grade 2/6 in intensity. It is transient, usually disappearing by six months of age.
2. A transient systolic murmur of PDA is heared at the upper left sternal border and the infraclavicular area on the first day, and it usually disappears shortly thereafter.
3. A transient systolic murmur due to tricuspid regurgitation (TR) is indistinguishable from that of VSD and is maximally audible at the lower left sternal border. This murmur is more common in infants who had fetal distress or neonatal asphyxia, because they tend to maintain a high PVR for a longer period.

Most pathologic murmurs should be audible during the first month of life. However, the time of appearance of heart murmur depends on the nature of the defect.

1. Heart murmurs of stenotic lesions, i.e. aortic stenosis, pulmonary stenosis and coarctation of the aorta, are audible after birth and persist because they are not affected by the degree of PVR.
2. Heart murmurs that depend on the reduced PVR, e.g. heart murmurs due to left-to-right shunt lesions, such as VSD. The onset of a loud VSD murmur may be delayed until 1–2 weeks of age.
3. The continuous murmur of a large persistent PDA. This is a crescendo systolic murmur with a slight or absent diastolic component.

Even in the absence of a murmur, a newborn infant may have a serious heart defect that requires immediate attention, for instance when there is transposition of the great arteries (TGA) or severe CHF, in which the murmur appears only when myocardial function has improved because of medical treatment.

75.7.4 Arrhythmias

For a detailed description of identification and management of neonatal arrhythmias, see Chapter 77.

75.8 Clinical Evaluation of the Neonate with CHD

Congenital heart diseases with early presentation account for a significant proportion of all neonatal emergencies; early diagnosis is vital to achieve correct treatment and favorable prognosis. Critical CHD includes all cardiovascular lesions that would result in neonatal death without immediate intervention to palliate or correct the anatomic defect. The initial assessment of the newborn with possible CHD should include timing of presentation and severity of symptoms. These depend on: (1) the nature and severity of the anatomic defects; (2) the impact of the alterations to normal physiology that occur during the first week of life with the closure of ductus arteriosus and fall of PVR; (3) intrauterine effects of the structural lesions.

75.8.1 Initial Evaluation

75.8.1.1 History

The history can provide useful information leading to the suspicion of CHD: (1) maternal disease during first trimester of gestation due to viral infections, X-ray exposure, drug intake, alcohol abuse, diabetes; (2) problems in the perinatal period (low Apgar score and/or the need of cardio-pulmonary resuscitation).

75.8.1.2 Physical Examination

Physical examination of the neonate can provide important clues to the anatomic diagnosis.

Inspection: to look for the presence of cyanosis with or without respiratory distress, respiratory pattern including the effort of breathing and use of accessory muscles. A general appearance typical of a syndrome, chromosomal abnormalities or malformations which may be associated with CHD.

Palpation: reduced or absent distal pulses are highly suggestive of obstruction of the aortic arch. Weak arterial pulses are characteristic of critical aortic valve stenosis, hypoplastic left heart syndrome or cardiogenic shock. Palpation of the precordium may detect the presence of a thrill that usually indicates at least moderate outflow tract obstruction or a restrictive VSD with low right ventricular pressure. Hyperdynamic precordium suggests heart disease with high volume overload such as left-to-right shunt lesions and/or severe valvular regurgitation. A cool neonate with prolonged capillary refill time should always raise the possibility of severe CHD.

Auscultation: it is rarely conclusive for CHD diagnosis. More than 50% of full-term newborn infants have an innocent murmur at some time during the first week of life (see § 75.7.3).

Most pathological murmurs should be audible during the first month of life. Heart murmurs audible immediately after birth are due to stenotic lesions (e.g. aortic stenosis, pulmonary stenosis, and coarctation of aorta), or left-to-right shunt lesions (e.g. a small VSD). The onset of a murmur due to a large VSD may be delayed until first or second week of life. The continuous murmur due to a PDA may not appear for two to three weeks. Diastolic murmurs are always indicative of cardiovascular disease. During the auscultation, the physician must pay attention to the heart rate, noting its regularity and/or variability.

75.8.1.3 Four-limb Blood Pressure

Blood pressure should be measure in the upper and lower limbs. A difference between arms and legs of more than 10 mmHg is suggestive of coarctation of the aorta, aortic arch hypoplasia or interrupted aortic arch. Blood pressure measurements are highly specific but not sensitive for the diagnosis. Moreover, coarctation cannot be ruled out even in absence of significant blood pressure gradient as it may occur when a large ductus arteriosus is still patent.

75.8.1.4 Chest X-ray

Chest X-ray can give important clues leading to the diagnosis of CHD and/or associated lung disease. The cardiothoracic ratio of normal newborn infants is > 0.5; the normal value in older children and adults is < 0.5. Therefore, the evaluation of heart size should take into consideration the degree of inspiration, judged by the level of the diaphragm. Particularly in newborns, the heart size may be difficult to determine due to an overlying thymus. In addition to the heart size, its position within the chest should be considered to assess visceral and cardiac situs (e.g. dextrocardia, right or left isomerism is frequently associated with CHD).

Cardiac silhouette may be typical in some CHD as the "egg shape" in transposition of the great arteries (Fig. 75.2).

Evaluation of pulmonary vascular markings in neonates can be difficult. It is often hard to distinguish between increased pulmonary blood flow and pulmonary venous congestion. A poorly perfused lung field suggests decreased pulmonary blood flow and indicates serious cyanotic congenital heart defects or significant left atrial hypertension.

In neonates with infracardiac total anomalous pulmonary venous drainage (which is almost always "obstructed") the chest-X-ray shows a small heart, upper lobe blood diversion, fluid in the fissures (signs of pulmonary edema). Differentiating between primary pulmonary disease and respiratory distress syndrome is sometimes difficult.

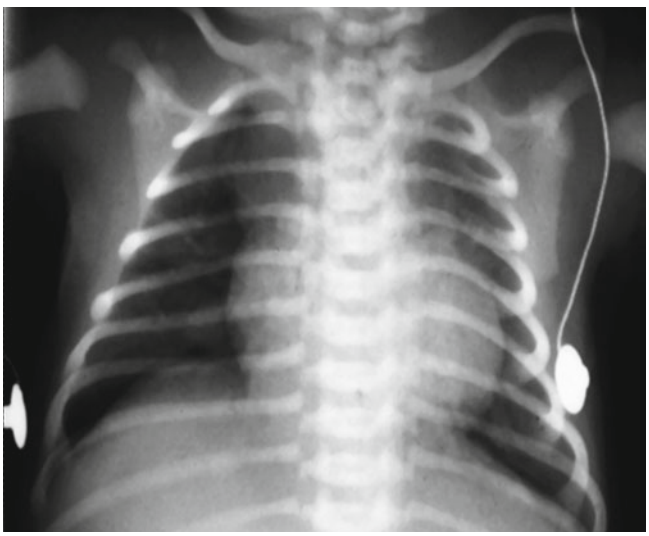

Fig. 75.2 Trasposition of the great vessels. The heart is enlarged and has an egg-shaped configuration

75.8.1.5 Electrocardiogram (ECG)

The normal ECG of a newborn is different from that of an older child because it reflects the hemodynamic relations existing in utero. Usually it shows: (1) ainus tachycardia with a rate as high as 160 beats/minute; (2) right-sided deviation of the QRS axis with a mean of +125 degrees and a maximum of +180 degrees; (3) relatively small QRS complex voltages and T wave; (4) right ventricular dominance with tall R waves in V4R, V1 and V2; (5) occasional Q waves in V1. Diagnostic value of ECG in the newborn period is highly non specific and the precise diagnosis of CHD is generally obtained by other diagnostic tools, in particular echocardiography.

75.8.1.6 Hyperoxia Test

In newborns with suspected CHD, a hyperoxia test should be considered to make the differential diagnosis between oxygen desaturation due to heart disease and that due to pulmonary disease. Although this test does not give a complete diagnosis, it remains useful, especially when an echocardiogram is not easily or quickly available. The hyperoxia test is obtained by measuring the oxygen tension in room air (if tolerated), followed by repeated measurements with the patients receiving 100% inspired oxygen for 5 minutes. If possible, the arterial partial pressure of oxygen (PO_2) should be measured directly by arterial puncture, although PO_2 values obtained from a properly applied transcutaneous oxygen monitor (TCOM) are also acceptable. The measurements should be taken at both "preductal" and "postductal" sites.

- When a baby breathes 100% oxygen, an arterial PO_2 of 250 mmHg in both upper and lower limbs virtually eliminates critical structural cyanotic heart disease (positive test).

- Patients with pulmonary disease only will show a significant increase in PO_2.
- Stable intracardiac right-to-left shunting (failed or negative test). An arterial PO_2 of <100 mmHg in absence of lung disease is most likely due to intracardiac right-to-left shunting. There is only a mild increase of oxygen saturation, but it is usually below basal levels.
- Patients with large left-to-right shunting and systemic hypoxemia. In this situation there is a high level of oxygenation, because the inspired oxygen normalizes the saturation of pulmonary venous blood, which can be low due to pulmonary edema and oxygen diffusion gradient (an example of this situation is represented by an unobstructed total anomalous pulmonary venous return).
- Patients with PO_2 between 100 and 250 mmHg may be affected by a structural cardiac lesion with intracardiac mixing and greatly increased pulmonary blood flow as seen when there is a single ventricle.
- Markedly higher oxygen content in the upper versus the lower part of the body. This is an important diagnostic clue to lesions that include all forms of critical aortic arch obstruction or left ventricular outflow tract obstruction.
- Reverse differential cyanosis. This occurs in neonates with transposition of the great arteries and those with abnormal pulmonary to aortic shunting due to coarctation, interruption of the aortic arch, or suprasystemic pulmonary vascular resistence (persistent fetal circulation).

Patients with a positive hyperoxia test are likely to have a duct dependent CHD and prostaglandin E_1 should be given, until anatomic abnormality can be defined.

75.8.1.7 Stabilization and Transport

After the initial evaluation, attention should continue to be focused on the basic neonatal life support and maintaining patency of ductus areriosus. Airways should be stabilized, vascular access secured, volume status, and inotropic support maintained and systemic and pulmonary circulation kept in balance. The neonate may need to be transferred to a medical center with full pediatric cardiological facilities.

75.8.1.8 Prostaglandin E_1

When systemic perfusion depends on patency of the ductus arteriosus, a continuous infusion of PGE_1 may be lifesaving. PGE is indicated for the treatment of ductal dependent lesions. Indications and treatment recommandations are reported in Tables 75.3 and 75.4.

The mechanism of PGE_1 action consists of relaxation of the smooth muscle fibers forming the ductal tissue. In other tissues PGE_1 has been shown to result in increased levels of nitric oxide and nitric oxide syntethase [2]. When ductal dilatation occurs, systemic perfusion increases (peripheral vasodilatation) and pulmonary congestion may decrease. This change is accompanied by a rise in PH and a decrease in lactate accumulation because of improved tissue perfusion.

Table 75.3 Indications for prostaglandin E_1

Cyanotic heart disease with severely limited or no pulmonary blood flow
- Tricuspid atresia
- Pulmonary atresia and critical pulmonary stenosis

Children with obstructed systemic blood flow may depend on ductal patency, e.g. in the following conditions
- Aortic atresia
- Critical aortic stenosis
- Critical coarctation of the aorta
- Interrupted aortic arch
- Hypoplastic left heart syndrome

Patients with transposition of great arteries benefit from patency of the ductus arteriosus because the pulmonary and systemic circulation is in parallel.

Table 75.4 Recommandations for treatment with prostaglandin E_1

- The usual starting dose is 0,05 mcg/kg/min
- When the desired effect is achieved, reduce to 0.025 mcg/kg/min
- Secure vascular access

Adverse effects include respiratory depression; PGE can cause apnea in 10–12% of the neonates and a stable airway must be maintained. Generally all neonates receiving PGE_1 infusion should be intubated for transport. Systemic hypotension and bradycardia have also been seen. Sometimes additional side effects are observed, e.g. bradycardia (7%), tachycardia (3%), edema and cardiac arrest (1%) [4]. PGE_1 inhibits platelet aggregation and may impair clotting. Prolonged use of PGE_1 has been associated with gastric obstruction and cortical hyperostosis of the long bones. Patients receiving PGE_1 need to be monitored for vital signs, including pulse oximetry, and arterial blood gas analysis.

Following the initial diagnosis and medical stabilization, the neonate can be transferred to a tertiary pediatric cardiac center fully equipped for any required additional treatment.

75.8.2 Confirming the Diagnosis

To confirm the diagnosis, two-dimensional and color Doppler echocardiography is used to define the anatomy, physiology and myocardial function.

Echocardiography is the most commonly used non-invasive diagnostic tool. It is harmless, but some precautions have to be taken if the patient is very sick: for example temperature stability should be maintained, extension of the neck for suprasternal views of the arch may be problematic, particularly in neonate with respiratory distress. Careful specialized nursing assistance is recommended.

Table 75.5 Indications for neonatal interventional catheterization

– Atrial septostomy (Rashkind)	
– Balloon valvuloplasty	Pulmonary valvular stenosis
	Aortic valvular stenosis
– Balloon angioplasty of native coarctation of the aorta	
– Transcatheter radiofrequency perforation and valvuloplasty	→ Atretic pulmonary valve
– Miscellaneous	Coil embolization of abnormal vascular communications
	Stenting of a PDA
	Stenting of the right ventricle outflow

75.8.2.1 Cardiac Catheterization

Cardiac catheterization is reserved for special cases where echocardiography is not diagnostic, as in definition of the anatomy of peripheral pulmonary branches, aorto-pulmonary collaterals, some complex pulmonary venous anomalies or in other situations where the decision making process can be hampered by an incomplete or incorrect diagnosis. Echocardiography is fully diagnostic for intracardiac anatomy, in virtually all the cases.

Interventional catheterization: nowadays, a wide range of interventional procedures can be carried out in very small babies (Table 75.5). These procedures include a pre-operative palliation, for instance by balloon atrial septostomy, or an alternative to surgery, e.g. by balloon dilatation of a pulmonary or aortic stenosis. Interventional catheterization can also play a role in a wide spectrum of postoperative sequelae, such as residual pulmonary branch stenosis. See the following text for further details on catheter interventional procedures.

Cardiac catheterization of newborns, either interventional or diagnostic, carries more risks than when performed on children. Basic medical stabilization, airway management, sedation and body temperature control are crucial. The baby's hemodynamic condition should be as stable as possible. Intravenous lines should be secured and intravenous infusion pumps adequately prepared. For example a prostaglandin infusion should be given by a dedicated intravenous line; flushing of this line should be avoided because bolus injection can cause apnea and hypotension.

75.9 CHD in the Newborn

The types of CHD that may present during the newborn period differ from those recognized later in infancy. During the neonatal period, CHD can be classified according to anatomy, physiopathology or timing of presentation. In each of the following sections, specific lesions will be discussed focusing on pathophysiology and clinical presentation.

75.9.1 Duct-Dependent Pulmonary Blood Flow

This group of CHD is characterized by obstruction of pulmonary blood flow due to severe pulmonary stenosis or pulmonary atresia. In this situation pulmonary blood flow depends mainly on the patency of the duct (Table 75.6).

75.9.1.1 Critical Pulmonary Valve Stenosis

Congenital obstruction to RV outflow can be classified as valvar, subvalvar or involving pulmonary trunk and peripheral pulmonary artery branches. This pathology (pulmonary stenosis, PS) is relatively common; the frequency approaches 8–10% of all cardiac defects. There are many anatomical descriptions about the pulmonary valve: dome-shaped valve; dysplastic hypertrophy of the pulmonary valve leaflets; unicommissural, bicuspid, tricuspid valve; hypoplastic annulus with dysplastic valve [5, 6]. The main cardiac consequence of PS is an elevated RV pressure because of obstruction to right ventricular cardiac output. The rise in pressure causes RV hypertrophy which is proportional to the degree of obstruction. This hypertrophy is particularly evident in the infundibular region, producing dynamic narrowing of the outflow tract. Because the ventricular septum is intact, the systolic pressure generated in the RV may exceed the LV systolic pressure. Moreover, the higher diastolic pressure in the RV caused by muscular hypertrophy results in a non compliant RV and increased right atrial filling pressure. When the right atrial pressure exceeds the left atrial pressure, a right-to-left shunt at the foramen ovale appears, resulting in central cyanosis or hypoxemia. There may be associated RV dysfunction and/or tricuspid regurgitation.

In critical pulmonary stenosis or pulmonary atresia the pulmonary flow is duct-dependent, as previously explained.

Newborns with critical PS present within the first week of life with cyanosis and hypoxemia and signs of CHF with peripheral vasoconstriction. Cardiac auscultation reveals a single second heart sound. The systolic murmur of PS may be soft or even absent, reflecting diminished flow across the right ventricle outflow tract. More often, a systolic regurgitant murmur due to tricuspid insufficiency is heard at the left midsternal border.

ECG reveals RV hypertrophy and right axis deviation.

Chest X-ray reveals reduction of pulmonary blood flow. Normal or severe cardiomegaly is present, as in case with severe tricuspid regurgitation.

Table 75.6 Duct-dependent pulmonary blood flow

Critical pulmonary valve stenosis
Pulmonary atresia with intact ventricular septum
Severe tetralogy of Fallot
Complex congenital heart disease with severe pulmonary stenosis or pulmonary atresia

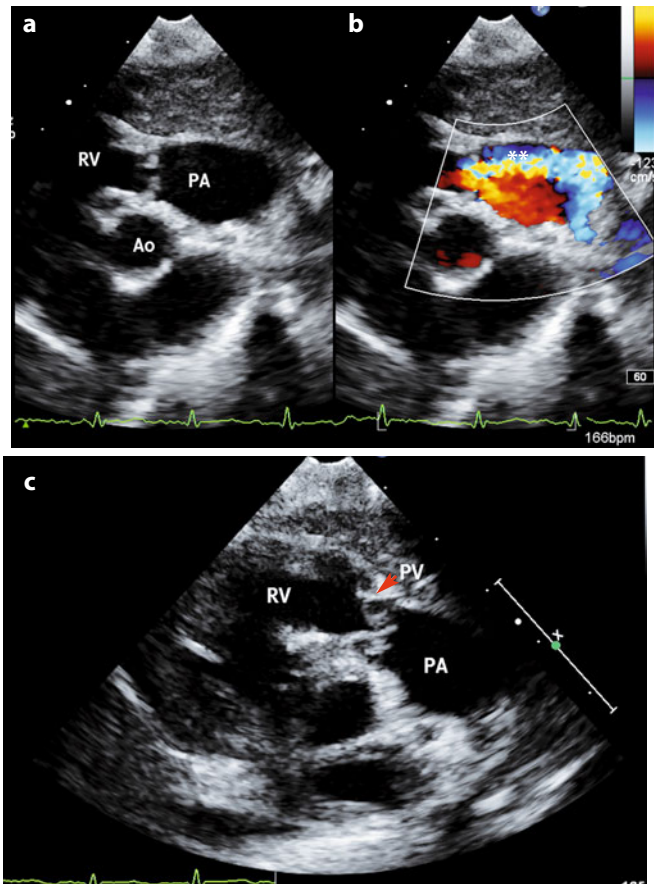

Fig. 75.3 Two dimensional echocardiogram in newborn with severe pulmonary valve stenosis. **a** A parasternal right ventricular outflow tract view demonstrating a systolic doming of the pulmonary valve leaflets. **b** Two-dimensional color Doppler echocardiogram (parasternal long axis) demonstrating a central jet (*asterisks*) across the pulmonary valve. The pulmonary trunk is dilated. *Ao* Aortic valve; *RV* right ventricle. **c** Thickened pulmonary valve cusps (*arrow*)

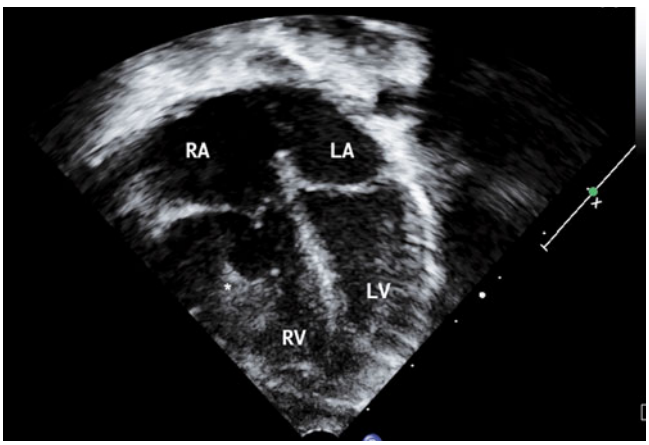

Fig. 75.4 Two-dimensional echocardiogram (apical four-chamber view, anatomical orientation) demonstrating a significant right ventricle hypertrophy. *RV* right ventricle; *RA* right atrium; *LA* left atrium; *LV* left ventricle

Differential diagnosis during the neonatal period: TGA, pulmonary atresia with intact ventricular septum, Ebstein's anomaly. The definitive diagnosis is made by echocardiography (Figs. 75.3 and 75.4)

75.9.1.2 Pulmonary Atresia with Intact Ventricular Septum

Pulmonary atresia with intact ventricular septum (PA-IVS) is a rare lesion occurring in an estimated 8 of 1000 live births [6].

PA-IVS is characterized by abnormalities proximal to the RV-PA junction. This pathology includes a spectrum of disease ranging from imperforate pulmonary valve with a well-developed RV to a severely hypoplastic-rudimentary RV with RV coronary fistulae and absent infundibulum. Focusing on pathophysiology, in the case of rudimentary RV, systemic venous blood, brought to the right atrium from caval veins and coronary sinus, passes mainly into the left side of the heart across the foramen ovale without passing the lungs. Anatomically, the pulmonary valve is often well formed with definable fused commissures. There is also a form when the valve is more dysmorphic with unicuspid or quadricuspid configurations, as well as complete absence of some valvular architecture with a fibrous ventriculoarterial junction [7]. Typically, the tricuspid valve (TV) is smaller than normal. The TV is commonly dysplastic and variably regurgitant. The size of the TV correlates with the RV cavity size, and this is important in the prognosis of PA-IVS. The RV is hypertrophied with a small cavity in 90% of cases and severely hypoplastic in more than 50%. RV may be unipartite (inlet only), bipartite (inlet and outlet), or tripartite (inlet, outlet and trabecular portions). Coronary artery abnormalities are common, including areas of stenosis or complete atresia. Retrograde blood flow from the hypertensive RV into coronary circulation through ventriculo-coronary connections (sinusoids) may be necessary for myocardial perfusion (Fig. 75.5). Ventriculo-coronary fistulae are not present in cases of significant tricuspid regurgitation, presumably because RV hypertension does not develop. After birth, with ductal runoff, diastolic pressure is relatively low and in competition with the suprasystemic RV pressure for coronary artery perfusion. The pulmonary arteries are usually confluent, supplied by a left-sided ductus arteriosus. Usually, because of the obligatory right-to-left shunt at the atrial level, these patients have a foramen ovale or an atrial septal defect (ASD).

Newborns with this malformation become cyanotic and hypoxemic immediately after functional closure of the PDA. When the ASD is restrictive, the cardiac output may be affected. Cyanosis is usually apparent within hours of birth, worsening progressively. In the absence of significant acidosis and reduced cardiac output, tachypnea may be evident. Cardiac examination reveals single first and second heart sounds. A soft pansystolic murmur is often audible at the left lower sternal border. Sometimes the murmur of TR is prominent.

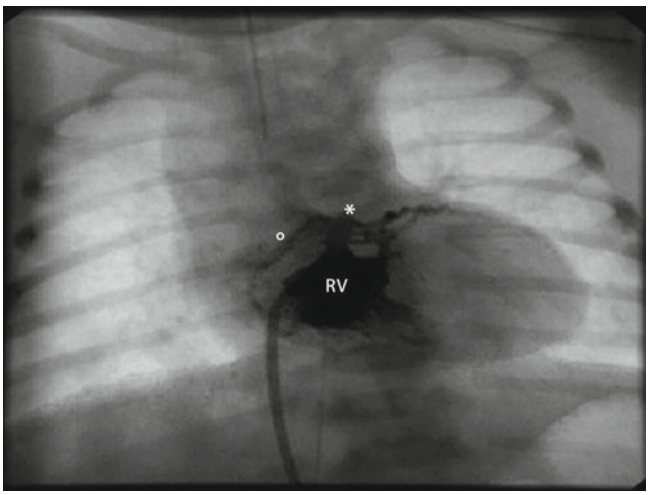

Fig. 75.5 Angiogram of a newborn with pulmonary atresia with intact ventricular septum. The right outflow tract (*asterisk*) ends blindly at the level of the atretic pulmonary valve (*circle*). Coronary sinusoids. *RV* right ventricle

Chest X-ray reveals a variable degree of cardiomegaly. Pulmonary vascular markings are decreased.

ECG reveals an LV dominance or significant LV hypertrophy. There is right atrial enlargement and ST-T abnormalities, consistent with some degree of subendocardial ischemia.

Echocardiography is effective in making the diagnosis. Catheterization is indicated for precise assessment of possible presence of sinusoids, RV to coronary fistulae, stenosis of the coronary vessels when there is a severely hypoplastic RV ("RV dependent coronary circulation"). However, when there is a tripartite RV, perforation of the atretic pulmonary valve followed by balloon dilation can be performed as an alternative to surgical valvulotomy.

75.9.1.3 Tetralogy of Fallot

Tetralogy of Fallot (TOF) occurs in 3–6 infants per 10,000 live births [8]. The etiology is multifactorial but reported associations include untreated maternal diabetes, phenylketonuria and an increased intake of retinoic acid. Some associated chromosomal anomalies have been described: trisomies of chromosomes 21, 18 and 13; however, the most frequent association is with 22q11 chromosome microdeletion [9]. This deletion, manifested by varying degrees of palatal abnormalities, dysmorphic facies, learning difficulties, immune deficiencies and hypocalcemia, is typical of DiGeorge syndrome.

Anatomically, TOF results from anterior and cephalad deviation of the infundibular portion of the ventricular septum. This deviation causes: (1) aortic overriding over the crest of interventricular septum; (2) a malaligned subaortic ventricular septal defect; (3) obstruction of right ventricular outflow tract (RVOTO) (the level of the obstruction can be subvalvular, valvular, supravalvular, in the pulmonary artery or its branches, and either isolated or in combination); and (4) systemic pressure in the RV with secondary hypertrophy (Fig. 75.6).

In case of TOF with pulmonary atresia (PAVSD), the right and left pulmonary artery are confluent in approximately 50%, with blood to the pulmonary arteries flowing through the PDA. In the other 50%, pulmonary supply is multifocal. In these patients the pulmonary blood supply may originate partly from the "true" pulmonary arteries and partly from aorto-pulmonary collateral arteries (MAPCAS). If the pulmonary arteries are discontinuous or absent, pulmonary supply is only from MAPCAS or from a combination of MAPCAS and PDA.

Other associated lesions include atrial septal defects, additional ventricular septal defects and anomalous origin of coronary arteries. A right aortic arch is present in one-quarter of patients with TOF.

The initial presentation of TOF depends on the severity of obstruction of the right ventricular outflow tract.

Neonates with the most severe forms of TOF present with marked cyanosis. Pulse oximetry is equal in all limbs and demonstrates oxygen saturation of 70–80% in air. Arterial blood gas analysis demonstrates hypoxemia. Patients with less severe variants present during the neonatal period with mild-to-moderate cyanosis, but typically without respiratory distress.

The neonatal ECG usually shows right ventricular hypertrophy and right QRS axis deviation. A chest X-ray classically shows a small or absent main pulmonary artery with decreased pulmonary blood flow and, over time, a "boot-shaped" heart silhouette.

Diagnosis is confirmed by echocardiography and cardiac catheterization is not usually required.

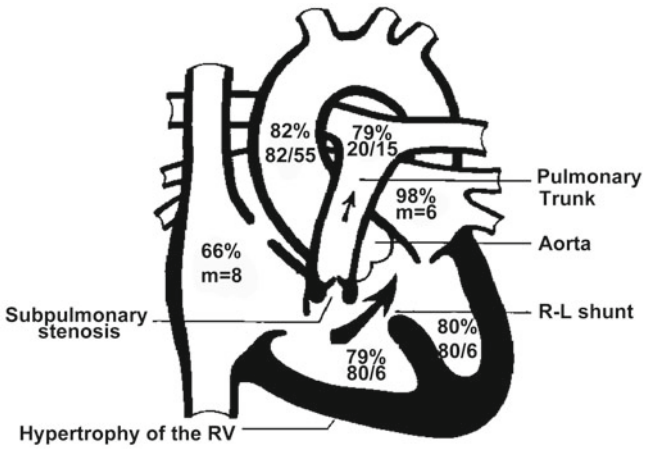

Fig. 75.6 Schematic diagram of oxygen saturation (%) and pressures in anatomy of Tetralogy of Fallot. The findings include: equal right and left ventricular pressures; a right-to-left (R-L) shunt at ventricular level; an anteriorly displaced infundibular septum resulting in subpulmonary stenosis

75.9.1.4 TOF with Absent Pulmonary Valve

Approximately 2–6% of patients described as TOF also have absent pulmonary valve leaflets [10]. The presence of rudimentary valvar leaflets results in free pulmonary regurgitation throughout fetal life. This chronic phenomenon produces chronic RV overload which is transmitted to the pulmonary arteries, with concomitant dilatation of these vessels.

There may be cyanosis in a neonate when the RV pressure is relatively high and when relatively low compliance of the RV decreases the antegrade flow towards the pulmonary circulation and causes right-to-left shunting through the VSD. As the RV pressure decreases, the cyanosis becomes less evident, and more left-to-right shunt develops. Congestive heart failure signs might appear when PVR drops, causing an increased pulmonary blood flow. Some babies can present with inspiratory and expiratory stridor due to tracheobronchial tree compression by aneurysmal pulmonary arteries. In most of these cases PDA is absent.

75.9.1.5 Severe Ebstein's Anomaly

Ebstein's anomaly (EA) is an uncommon but severe anatomic lesion when it presents in the neonatal period. Some familial cases have been reported. Overall, recognized EA is rare, accounting for less than 1% of CHD [11].

Ebstein's anomaly is characterized by abnormal attachment of the posterior and septal tricuspid leaflets in the RV cavity far from a normally positioned tricuspid valve annulus. The degrees of leaflet displacement are variable. The anterior leaflet is large, often with abnormal cordal attachments. This leaflet may obstruct the RV outlet. The inlet portion of the RV is usually functionally integrated into the right atrium ("atrialization of the RV") and the functional RV cavity is small. Nearly all patients have a coexisting ASD or PFO. Ebstein's anomaly can be associated with accessory atrioventricular conduction pathways (Wolff-Parkinson-White syndrome).

Clinical presentation depends on the type and severity of EA. At birth, pulmonary vascular resistance is high and the degree of cyanosis can vary with the degree of right-to-left shunting at the atrial level. In neonates with relatively mild tricuspid valve displacement and no RV outflow tract obstruction, right-to-left shunting usually decreases over the first weeks of life due to pulmonary vascular resistance decrease, with a concomitant reduction in cyanosis. If there is a significant portion of RV atrialized or if the tricuspid valve anterior leaflet obstructs the outflow tract, cyanosis persists and the severity of CHF increases. Ebstein's anomaly is one of the few disorders that may cause a thrill in a neonate.

There are several characteristic ECG appearances: right QRS axis deviation, right bundle branch block, low-voltage QRS complex in the right precordial leads, right atrial enlargement, and first degree AV block. Arrythmias can occur in more than 50% of patients; most frequently they are tachyarrhythmias, including paroxysmal supraventricular tachycardia, atrial fibrillation and atrial flutter.

Cardiac size may vary from nearly normal to extreme cardiomegaly, which is rather typical of EA.

75.9.1.6 Complex CHD with Severe Pulmonary Stenosis or Pulmonary Atresia

Some patients with tricuspid atresia, single ventricle and transposition of the great arteries may have severe pulmonary valve stenosis or pulmonary valve atresia. Postnatally, the degree of restriction to pulmonary flow will determine the clinical presentation and physical findings. Most babies present with cyanosis or murmur. In some of these neonates restrictive ASD may be an important problem, especially in neonates with single ventricle and atrioventricular valve stenosis.

75.9.2 Duct-Dependent Systemic Blood Flow

In this group there is a unique physiology related to left ventricular outflow obstruction (LVOTO) characterized by total or partial arterial duct dependent systemic perfusion (Table 75.7). During intrauterine development, systemic perfusion is not compromised, being maintained by the ductal flow from the right ventricle to aorta. Postnatally, acute impairment of systemic perfusion follows ductal constriction. When the PDA closes, infants present with cardiovascular collapse and symptoms of CHF.

75.9.2.1 Aortic Stenosis

The normal aortic valve has three leaflets of approximately equal size with a semilunar shape at the level of the free margins. Abnormalities of the aortic valve account for 70–80% of left ventricular outflow tract obstruction. In severe neonatal aortic stenosis (AS) the valve is often unicuspid or bicuspid (Fig. 75.7).

During fetal development, normal growth and development of the fetus is generally maintained in spite of valvar aortic stenosis. LVOTO exposes the left ventricle to increased

Table 75.7 Lesion-specific: ductus arteriosus dependent systemic blood flow

– Aortic stenosis
– Coarctation of the aorta
– Interrupted aortic arch
– Hypoplastic left heart syndrome

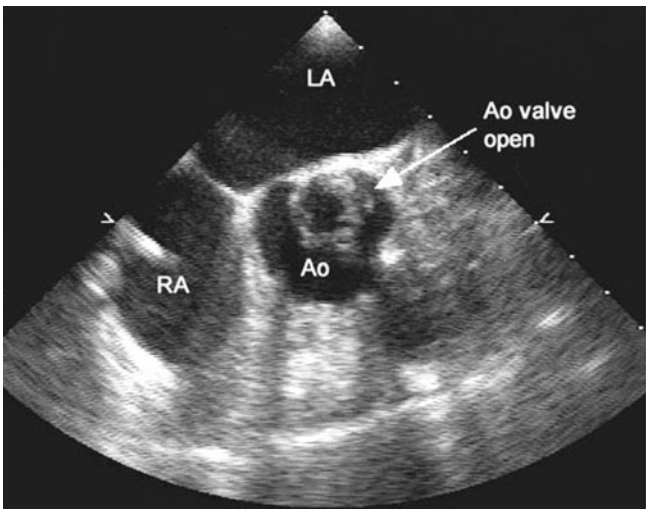

Fig. 75.7 Two dimensional echocardiogram (parasternal short axis) demonstrates an uncuspid and thickened aortic valve

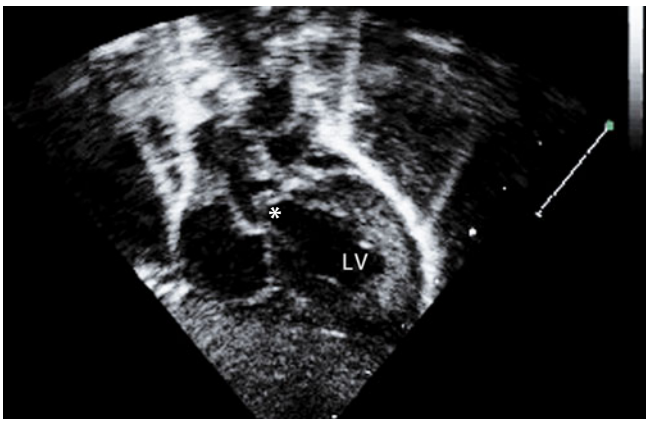

Fig. 75.8 Subcostal coronal view: Neonate with aortic valvar stenosis, concentric left ventricle (LV) hypertrophy. Aortic valve (*asterisk*)

afterload and results in ventricular hypertrophy and myocardial dysfunction. Moreover, chronic in-utero subendocardial ischemia secondary to hypertrophy and increased intracavitary pressure can lead to coronary ischemia and development of endocardial fibroelastosis which further impairs the ventricular function. Additionally, reduced antegrade blood flow through the aortic valve will predispose to underdevelopment of the left heart structures and hypoplasia of the mitral valve, left ventricle, and aortic arch. The RV supplies an increased proportion of systemic blood flow via the PDA because LV output is reduced.

Neonates with critical aortic stenosis present dramatically soon after birth. As the ductus arteriosus begins to close, severe systemic hypoperfusion occurs resulting in decreased coronary perfusion, acute hemodynamic deterioration with cardiovascular collapse, acidosis, organ injury and shock. Once the baby is stabilized, a complete echocardiographic evaluation is required to assess the severity and levels of the obstruction, associated cardiac abnormalities and related hypoplasia of the aortic arch and left heart structures.

Neonates and infants with less severe obstruction may present with failure to thrive and tachypnea due to increased work of breathing in association with pulmonary vascular congestion due to left atrial hypertension.

ECG findings vary widely in newborns and infants with AS. The right ventricular hypertrophy is the predominant pattern in neonates.

Chest X-ray reveals global cardiomegaly in most of the cases. Pulmonary vascular markings are usually normal, but in 30–50% of cases congestion may be evident.

Following the anatomic definition by echocardiography (Figs. 75.8 and 75.9) treatment strategy depends on: (i) the degree of aortic stenosis and the presence of aortic regurgitation; (ii) associated cardiac lesions; (iii) the severity of lesions involving other organs e.g necrotizing enterocolitis.

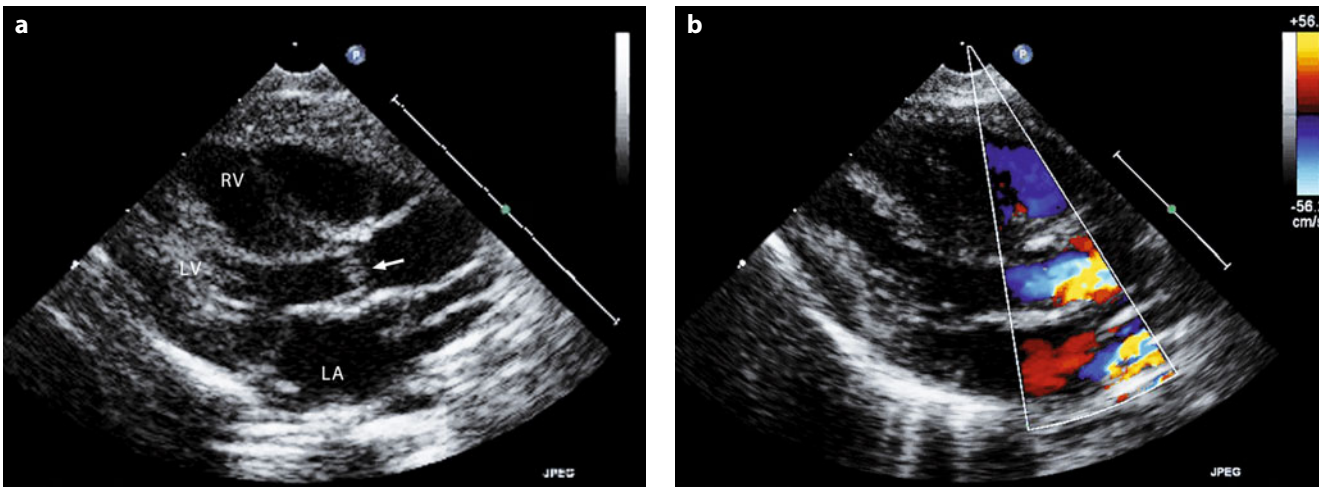

Fig. 75.9 Parasternal long-axis view in systole. **a** The valve has a domed appearance, with thickening of cusps (*arrow*). *LA* left atrium; *RV* right ventricle; *LV* left ventricle. **b** Color Doppler demonstrating a turbulent flow across the aortic valve

75.9.2.2 Coarctation of the Aorta

Coarctation of the aorta (CoAo) occurs in 8–10% of all cases of CHD. It is a hemodynamically significant narrowing of the aorta located mostly at the insertion of the ductus arteriosus. It could be isolated or associated with other abnormalities, e.g. tubular hypoplasia of the aortic arch, left ventricular outflow obstruction, bicuspid aortic valve, VSD and ASD. CoAo has been classified as preductal, ductal and postductal. It is frequently seen in some chromosomal syndromes such as Turner syndrome. In newborns or young infants, the presentation is often dramatic as shock or severe congestive heart failure. A concomitant large PDA may make the diagnosis difficult. The most common signs and symptoms are: tachypnea, diaphoresis at feeding, poor perfusion, hepatomegaly and metabolic acidosis. If the duct is partially open, preductal saturation is higher than postductal saturation; there is upper limb hypertension and weak palpable femoral pulses. If the duct is completely closed, there is no discrepancy between upper and lower limb saturation, femoral pulses are absent, cyanosis or lower limb mottling and upper limb hypertension are present.

CoAo and absence of four limb systolic pressure gradient can occur in the following anatomical and physiological conditions. (i) The duct is still patent, so the RV provides flow to the lower part of the body; these neonates have a differential cyanosis with lower O_2 saturation taken from lower limbs. (ii) LV dysfunction and systemic hypotension so severe that it becomes impossible to detect a pressure gradient. (iii) Aberrant right subclavian artery arising distally to the coarctation site; therefore right arm pressure reflects post-coartctation pressure.

The key point is not to exclude diagnosis of CoAo solely through the absence of a gradient in pulse volume or systolic pressure between upper and lower limbs.

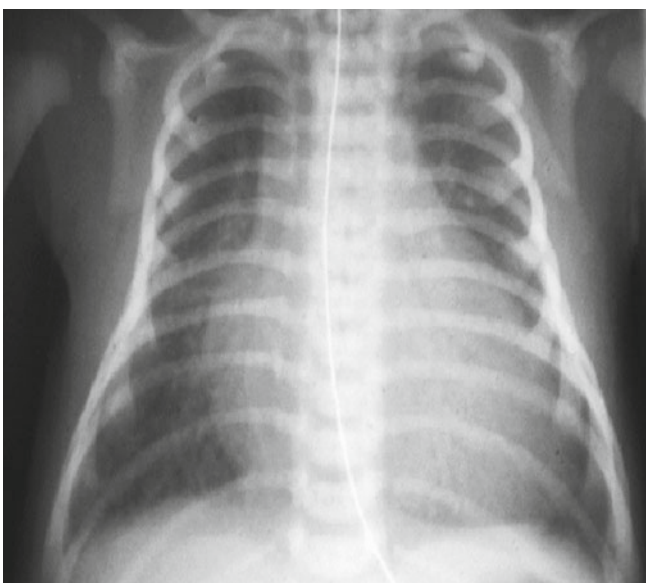

Fig. 75.10 Severe coarctation of the aorta. Heart is enlarged. Pulmonary arterial markings are normal, but there are distended pulmonary veins

The most common ECG pattern shows right ventricular hypertrophy initially, while biventricular hypertrophy usually appears later. Chest X-ray reveals the hallmarks of cardiomegaly and pulmonary congestion (Fig. 75.10). Echo color Doppler is the most sensitive and specific method for making the diagnosis (Figs. 75.11 and 75.12).

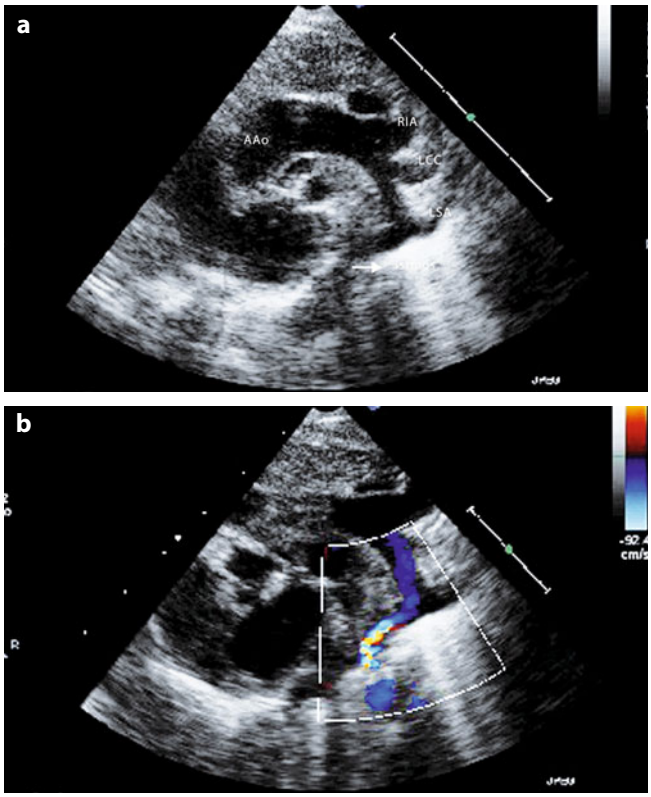

Fig. 75.11 Two dimensional echocardiography of a typical coarctation viewed from the high left sagittal plane. **a** The aortic coarctation is visualized distal to the origin of the left subclavian artery (LSA). Ascending aorta (AAo); right innominate artery (RIA); left common carotid (LCC). **b** Color flow picture showing the isthmic coarctation

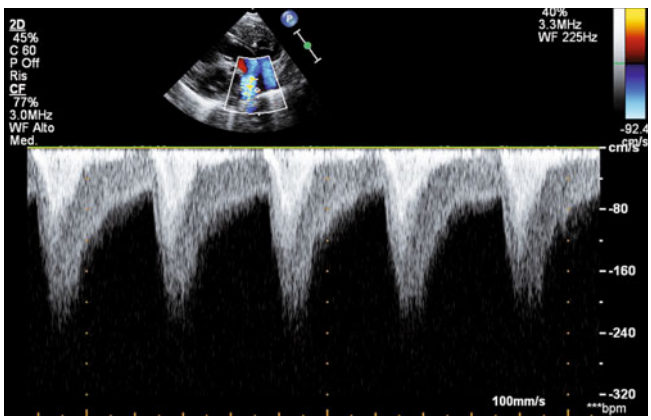

Fig. 75.12 Doppler sample: A continuous wave Doppler pattern at the level of the coarctation showing significant diastolic tail

Table 75.8 Associated anomalies with interrupted aortic arch

– VSD with posterior malalignment
– Left ventricular outflow tract obstruction
– Truncus Arteriosus
– Transposition of great arteries
– Double outlet right ventricle
– DiGeorge syndrome

75.9.2.3 Interrupted Aortic Arch

Interrupted aortic arch (IAA) is an uncommon congenital cardiovascular malformation. The incidence is 1% of all congenital heart diseases, carrying a mortality rate higher than 90% in the neonatal period [12].

IAA is defined as either a complete discontinuity or nonpatent fibrous strand in the trasverse arch or aortic isthmus. It is classified into three types according to the site of interruption: type A, the interruption is distal to the left subclavian artery origin; type B, between the origin of left subclavian and left common carotid artery; type C, which is the rarest one, between the origin of the right brachiocephalic and the left common carotid artery.

Its hemodynamic condition is totally dependent on PDA patency that supplies the lower part of the body. Furthermore, most patients have only an isolated VSD but IAA can be associated with a variety of complex cardiovascular anomalies (Table 75.8) [13].

In most of the cases the pathophysiology of interrupted aortic arch is similar to critical CoAo in the newborn. Systemic blood flow is dependent on PDA patency. When the PDA closes, the baby develops poor perfusion, acidosis, shock and renal failure. On physical examination, tachycardia, systolic murmur, hepatomegaly and weak femoral pulses are present. Differential cyanosis between the upper and lower limbs is usually difficult to appreciate when there is a VSD. If there is a concordant ventriculo-arterial connection, the pulse oximetry may show higher oxygen saturation in the pre-ductal upper limbs compared with post-ductal lower limbs. If the great arteries are transposed, the oxygen saturation could be higher in the lower limbs (reversal differential cyanosis).

IAA type B is commonly associate with DiGeorge syndrome (broad nasal bridge, malar hypoplasia, narrow palpebral fissures, hypertelorism, low-set posteriorly rotate ears, retrognathia, small mouth, and submucosal palatal cleft, variable thymus and parathyroid deficiencies). These physical features are difficult to recognize in neonates, therefore all neonates with IAA should be genetically investigated to confirm DiGeorge syndrome. The major risk in these patients is graft-versus-host disease after blood transfusion with non-irradiated blood.

ECG and chest X-ray are not specific. Echocardiography is fundamental to make the diagnosis and to detect coexisting defects.

75.9.2.4 Hypoplastic Left Heart Syndrome

Hypoplastic left heart syndrome (HLHS) represents a group of defects characterized by varying degrees of underdevelopment of left sided heart structures. Most of the patients have combinations of aortic and mitral valve atresia or stenosis [14]. The severity is determined by the severity of outflow tract obstruction and the degree of aortic hypoplasia. This syndrome accounts for 1–3.8% of all CHD [15].

The LV is a rudimentary non pumping chamber; the pulmonary venous return goes to the RA through a foramen ovale or atrial septal defect, causing complete venous blood mixing. The RV maintains the pulmonary and systemic output in a parallel fashion. The pulmonary arteries receive blood from the RV, while cerebral and cardiac perfusion are supplied retrogradely through the arterial duct into the transverse arch and ascending aorta.

During fetal life, the PVR is higher than the systemic vascular resistance and the RV maintains normal perfusion in the aorta and placenta through the duct. After birth, there is reversal of vascular resistance in the two circuits, with systemic pressure being higher than pulmonary, and PDA closure occurs. Additionally, an important anatomic element is the presence of an appropriately restrictive interatrial communication. With pulmonary and systemic parallel circulation, the ratio of pulmonary to systemic flow (Qp:Qs) depends on the balance between pulmonary and systemic vascular resistances. At birth, PVR is elevated but eventually decreases even in children with HLHS, resulting in increasing pulmonary blood flow and increasing RV overload, to preserve adequate systemic output. As Qp:Qs increases, more saturated blood passes from the LA to the RA across a non restrictive ASD; consequently systemic oxygen saturation approaches normal values despite progressive clinical signs of CHF. This mechanism represents the most common cause of CHF in the first weeks of life [6]. A different clinical presentation occurs when the pathophysiology is characterized by reduced pulmonary blood flow secondary to a small or restrictive interatrial communication. These babies always present critically with deep cyanosis, marked tachypnea, metabolic acidosis, hypoxemia characterized by PaO_2 values of 20 mmHg or less, and cardiovascular collapse.

All infants with HLHS should be promptly commenced on a prostaglandin infusion to ensure ductal patency and sysytemic perfusion. Clinical stabilization includes endotracheal intubation, vascular access placement, correction of metabolic acidosis, and oxygen delivery. After the diagnosis is made by echocardiography, prompt left atrial decompression is necessary by a balloon transcatheter or surgical procedure (atrial septostomy).

Newborns with HLHS are generally full-term babies who initially appear well and healthy; only a few are affected by extracardiac anomalies, like structural brain malformations typical of chromosomal abnormalities [16, 17]. The magnitude and distribution of acquired vital organ dysfunction usually

relates to the circulatory impairment at the time of diagnosis. All the interventions in the preoperative period should be done in order to: (i) preserve ductal patency and (ii) set or maintain Qp:Qs ratio of 1:1.

Differential diagnosis should include all the other left-sided obstructive lesions characterized by arterial duct dependent systemic circulation (Table 75.7) and other non-structural cardiac diseases with shock-like state clinical presentation such as neonatal sepsis or neonatal myocarditis; these conditions can be easily differentiated by echocardiography.

75.9.3 Parallel Circulation/Trasposition of the Great Arteries

Transposition of the great arteries (TGA) is a common form of cardiac anomaly, accounting for approximately 75–80% of all congenital heart defects [6]. TGA is defined anatomically as concordant atrioventricular (AV) connection and discordant ventriculoarterial connection. In this setting the morphological RA is connected to the morphological RV which gives rise to all or most of the aorta; the morphological LA is connected to the morphological LV from which the pulmonary trunk emerges (Fig. 75.13) [18]. This condition is known as simple transposition. By contrast, complex transposition includes VSD, left ventricular outflow tract obstruction, aortic arch anomalies and anomalous venous return.

Ventricular septal defect is present in 20% of TGA [19, 20], typically in the outlet portion. Malalignment of the outlet septum deviated to the right results in variable degrees of PA overriding representing an anatomical entity called double-outlet right ventricle with subpulmonary ventricular septal defect (Taussig-Bing anomaly).

Left ventricular outflow tract obstruction can be demonstrated in over 30% of the patients with TGA [21]. Aortic arch obstruction is rare in TGA without VSD. Coronary anatomy may show different patterns [22]. In 10% of the cases, this cardiac defect is associated with other noncardiac malformations [23].

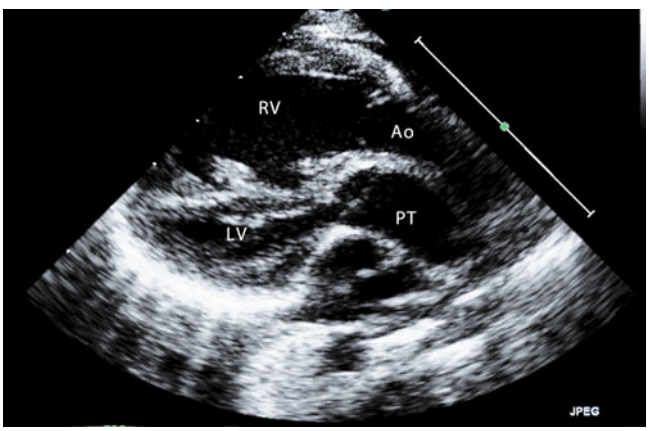

Fig. 75.13 Echocardiography; long-axis view demonstrating the anterior aorta arising from the right ventricle (RV) and the pulmonary trunk (PT) arising from the left ventricle (LV)

75.9.3.1 Transposition Physiology

Systemic blood goes from RA to RV and then into the aorta without being oxygenated in the lungs. On the contrary, fully oxygenated pulmonary venous blood returns from the pulmonary veins into LA and LV and returns to the lungs via the pulmonary artery: in this setting the systemic and pulmonary circulations describe parallel loops. Survival depends on the presence of one or more mixing sites between these two circulations such as atrial septal defect, ventricular septal defect or PDA, to achieve arterial oxygen saturation compatible with life.

Following separation of the placenta, newborns with TGA without VSD and small PFO or ASD have severe cyanosis (a very low PaO_2: 15–20 mmHg) on the first day of life, associated with high $PaCO_2$ and metabolic acidosis due to a severely decreased effective pulmonary blood flow.

75.9.3.2 Diagnosis

The hyperoxia test is suggestive of a cyanotic congenital heart condition [24]. Chest X-ray and ECG may be helpful, but findings are not specific. The definitive diagnosis relies on echocardiography.

The initial management aims at increasing FiO_2 and maximizing mixed venous oxygen saturation opening the arterial duct with prostaglandin. Resuscitation with mechanical ventilation can be necessary and the foramen ovale should be emergently enlarged by ballon atrial septostomy. This procedure is usually performed under fluoroscopy in the cardiac catheterization laboratory. Balloon atrial septostomy, also known as the Rashkind procedure, consists of placing a balloon tipped catheter in the LA, via the foramen ovale; the balloon is then inflated and pulled back into the RA, tearing the atrial septum. Echocardiographic guidance is mandatory. The aim of this procedure is to create an interatrial communication big enough to cause an increase in oxygen saturation due to improved blood mixing. In addition to these measures, medical support is usually necessary to optimize the clinical condition: correction of metabolic acidosis with hyperventilation and sodium bicarbonate to promote alkalosis, lower pulmonary vascular resistance and increase pulmonary blood flow.

In case of good atrial septostomy but persisting desaturation, oxygen saturation could be improved by: (i) decreasing the whole body oxygen consumption (muscle relaxants, sedation, mechanical ventilation); (ii) increasing cardiac output with inotropic agents; (iii) increasing oxygen-carrying capacity by treating anemia.

The arterial switch operation is the procedure of choice and it is used to achieve complete physiological and anatomical correction.

75.9.4 Lesions with Complete Intracardiac Mixing

75.9.4.1 Total Anomalous Pulmonary Venous Return

Total anomalous pulmonary venous return (TAPVR) is a congenital defect in which the pulmonary veins drain anomalously into the systemic venous circulation causing a complete blood mixing. TAPVR is classified in four types based on the anatomical sites of the abnormal connection: supracardiac, cardiac, infracardiac and mixed.

1. *Supracardiac*: the veins drain into a common confluence behind the LA which drain through an anomalous vein into the systemic venous circulation, usually in the right-sided superior vena cava or the innominate vein through a persistent vertical vein.
2. *Cardiac*: the common venous confluence drains either into the coronary sinus or posterior portion of RA.
3. *Infracardiac*: the common venous confluence passes across the diaphragm through the esophageal hiatus and drains either into the portal vein or ductus venosus or hepatic vein or inferior caval vein.
4. *Mixed type*: this anatomical configuration is the result of combination of different forms of TAPVR. It is commonly associated with other major cardiac anomalies.

In addition, drainage can be obstructed or unobstructed. The majority of supracardiac TAPVR cases have some degree of obstruction which could be secondary to: (i) ascending vein compression between bronchi and pulmonary artery or aorta, (ii) vertical vein orifice stenosis or (iii) a small and restrictive atrial communication.

Heterotaxy syndrome, especially right isomerism, is usually associated with TAPVR [25]. TAPVR is associated with other cardiac anomalies such as single ventricle, truncus arteriosus, transposition of the great arteries, pulmonary atresia and coartaction of the aorta [26].

There is an obligatory left-to-right shunt from pulmonary veins to the right heart and an obligatory right-to-left shunt from RA to the left heart to maintain the systemic output. The factors that determine blood flow distribution in these two circuits include: (i) relative size of the atrial communication and (ii) severity of any obstruction in the extracardiac pulmonary venous channel.

In the most common form of TAPVR, pathophysiology is a combination of pulmonary vein obstruction and increased pulmonary blood flow. This situation results in RV volume overload. Neonates with significant pulmonary vein obstruction present with severe cyanosis, tachypnea and hemodynamic instability. These babies are frequently misdiagnosed as having persistent fetal circulation or sepsis or severe respiratory distress syndrome [27].

The clinical presentation of TAPVR varies with the degree of obstruction and pulmonary blood flow. Frequently the newborns present with tachypnea due to RV volume overload, whereas babies with the important pulmonary vein obstruction show frank respiratory distress, pulmonary edema, poor peripheral perfusion, hypotension and metabolic acidosis. Identification of this pathology is crucial in critically ill neonates and the diagnosis is usually made by echocardiography.

75.9.4.2 Truncus Arteriosus

This defect consists of a single great artery arising from the heart which gives rise to systemic arteries, pulmonary and coronary arteries. Depending on the anatomical origin of the pulmonary arteries, four types of truncus arteriosus exist [28].

1. Type I, pulmonary trunk arises from common arterial trunk.
2. Type II, right and left pulmonary arteries arise separately but close to one another from the left postero-lateral aspect of common arterial trunk.
3. Type III, each pulmonary artery arises from the ipsilateral aspect of common arterial trunk.
4. Type IV, the main pulmonary artery is absent with the lungs receiving their blood supply through aortopulmonary collaterals.

The common arterial trunk overrides a VSD which is often large, nonrestrictive, with the superior border being formed by the truncal valve. The truncal valve may have a variable number of cusps, most commonly three; it is often anatomically abnormal showing different grade of regurgitation and/or stenosis. The pulmonary arteries are usually of normal caliber although stenosis of the origin and diffuse hypoplasia can occur. Coronary anomalies, in terms of origin and epicardial course, can coexist. Truncus arteriosus may also be associated with a right-sided aortic arch. The extracardiac anomalies are common and can contribute to the mortality; association with DiGeorge syndrome, chromosome 22q11 deletion [29], is reported in about 30% of cases [30].

In most cases the dominant physiology is a left-to-right shunt at the level of the great arteries. The pulmonary and the systemic blood flow is determined respectively by pulmonary vascular resistance and systemic vascular resistance. Newborns affected by truncus arteriosus generally have an elevated pulmonary vascular resistance and are therefore asymptomatic. As pulmonary resistance gradually decreases and PBF increases, signs of CHF occur. Subsequently the volume and pulmonary pressure overload leads to pulmonary vascular disease. Furthermore, signs of CHF can be worsened by concomitant significant truncal valve regurgitation or stenosis. Echocadiography provides accurate diagnosis (Figs. 75.14 and 75.15). Definitive repair of truncus arteriosus consists of PA branches separation, RV-PA conduit implantation and VSD closure.

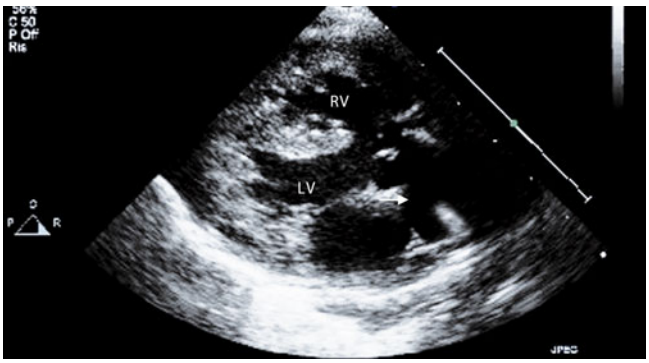

Fig. 75.14 Echocardiogram (long-axis parasternal view) of truncus arterious. Truncal vessel gives rise to the aorta and the pulmonary arteries (*arrow*). *RV* right ventricle, *LV* left ventricle

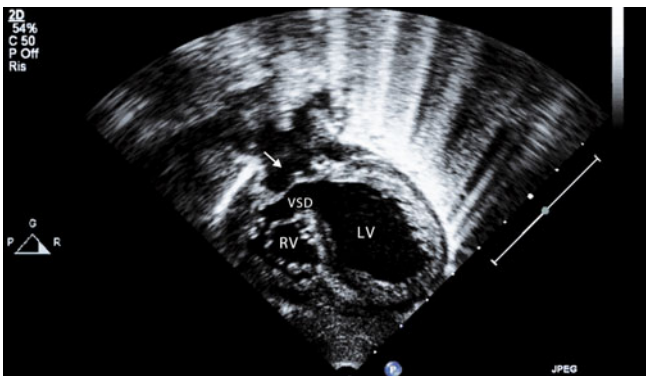

Fig. 75.15 Subcostal view demonstrating the ventricular septal defect (*VSD*) overriding the truncal valve. *RV* right ventricle, *LV* left ventricle

75.9.4.3 Complex Single Ventricle

Physiology of complex single ventricle is characterized by complete mixing of systemic and pulmonary venous circulation. An anatomical definition describes single ventricle as a malformation in which both atrioventricular valves enter one ventricle and there is only one identifiable ventricular sinus [31]. A large variety of anatomic lesions exist, generally associated with atresia of an atrioventricular or semilunar valve. The great vessels usually arise from the heart in an abnormal fashion; frequently variable degrees of pulmonary or aortic obstruction may be present. Most complex single ventricles are associated with heterotaxy syndrome: polysplenia or asplenia, systemic venous return anomalies, anomalous pulmonary venous connection, visceral situs abnormalities, atrioventricular septal defect.

The hemodynamic characteristics and the clinical presentation depend on the balance of anatomy and/or resistance in the systemic and pulmonary circulation. Patients without pulmonary stenosis show increased pulmonary blood flow and soon develop signs of congestive heart failure. In cases with pulmonary stenosis, the cyanosis is present according to the degree of osbstruction.

Patients with pulmonary or aortic atresia or severe hypoplasia of the aortic arch have a ductal-dependent circulation and they need treatment with PGE_1 as long as palliative surgical procedure is performed.

75.9.5 Left-to-right Shunt Lesions

In the group of left-to-right shunt lesions, the most common in the neonates are: PDA, ventricular septal defect, atrioventricular septal defect and atrial septal defect. PDA will be widely described in Chapter 80.

75.9.5.1 Ventricular Septal Defect

Ventricular septal defect (VSD), is one of the most common isolated congenital cardiac malformations. A ventricular septal defect is a hole in the interventricular septum resulting in direct communication between the left and right ventricles. A clinical useful classification schema divides VSD in four types, considering interventricular septum from the RV aspect:

1. Perimembranous, which partially or totally involves the membranous septum. It can extend into the inlet, trabecular or outlet portions of the septum. This accounts for the majority of the cases.
2. Subarterial, located just beneath the pulmonary valve. This defect, also known as "doubly committed", occurs most often amongst Asians.
3. Inlet defect, beneath the septal cusp of the tricuspid valve.
4. Muscular defect, located anywhere in the muscular septum with all muscular margins (Fig. 75.16).

VSD could be either isolated or part of complex CHD as in TOF, truncus arteriosus, double outlet RV.

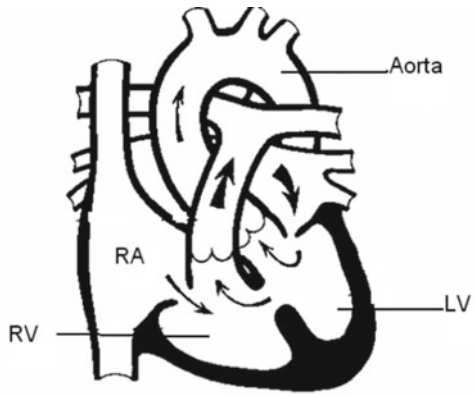

Fig. 75.16 Ventricular septal defect: VSD may occur anywhere in the ventricular septum. This defect is located in the muscular portion of the septum. *RV* right ventricle, *LV* left ventricle

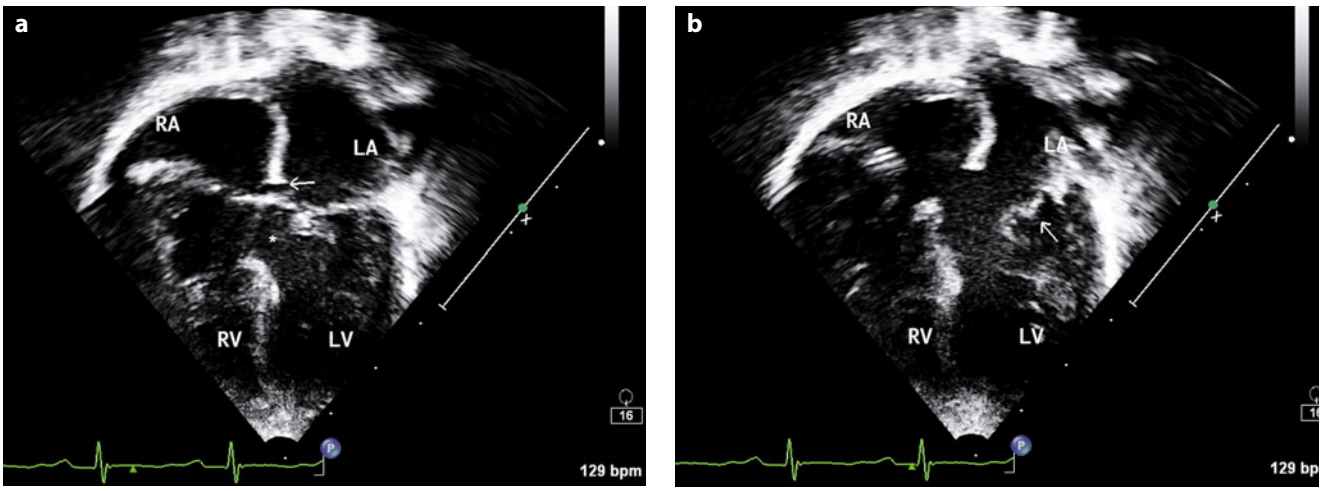

Fig. 75.17 **a** Two dimensional echocardiogram (apical four-chamber view, anatomical orientation) demonstrating ostium primun atrial septal defect. Inlet ventricular septal defect (*asterisk*). **b** Two dimensional echocardiogram (apical four-chamber view, anatomical orientation) demonstrating a complete atrioventricular septal defect. The common atrioventricular valve is indicated (*arrow*). *RA* right atrium, *LA* left atrium, *RV* right ventricle, *LV* left ventricle

75.9.5.2 Atrioventricular Septal Defect

Atrioventricular septal defect (AVSD) is a malformation involving the structures normally derived from the endocardial cushion tissue: ostium primum atrial septal defect, inlet VSD and the common atrioventricular valve (Fig. 75.17). Different forms of AVSD exist but two are the most common: partial and complete. Partial AVSD indicates an atrioventricular septal defect with interatrial communication (ostium primum ASD), common AV valve but not inlet VSD; the term complete atrioventricular septal defect describes an AVSD with both ostium primum ASD and inlet VSD. There is a strong association between complete AVSD and Down syndrome whereas partial AVSD is more seen in DiGeorge and Ellis-Van Creveld syndromes.

Most neonates with left-to-right shunt defects present with signs of cardiac failure after 2 weeks of age. During fetal life even large defects do not have major physiological effects because of high PVR. After birth the normal decline of PVR occurs within the first 2 weeks. In the presence of a large shunt, this decline may be delayed by 1–3 months. When this phenomenon happens, pulmonary artery blood flow increases resulting in left heart volume overload.

Babies with small left-to-right shunt defects usually come to the attention of pediatricians because of cardiac murmurs without symptoms. Children with moderate left-to-right shunt defects show varying degrees of growth retardation. Large defects cause signs and symptoms of cardiac failure as tachypnea, nasal flaring, rib and sternal retraction, feeding difficulties, irritability, excessive sweating. It is extremely rare for partial AVSD to cause abnormal physical signs during neonatal period, unless there is significant AV valve regurgitation.

All the neonates with signs of cardiac failure should be investigated by echocardiography.

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76

Early Diagnosis of Congenital Heart Disease: When and How to Treat

Luciane Piazza, Angelo Micheletti, Diana Negura, Carmelo Arcidiacono, Antonio Saracino and Mario Carminati

76.1 Introduction

Cardiovascular malformation is the most common group of congenital malformations. The prevalence is 5.3 cases per 1000 live births [1–3]. These malformations represent a significant cause of neonatal morbidity and mortality. Advances in medical and surgical treatment of congenital heart disease (CHD) have resulted in a trend towards an improved outcome in conditions which previously carried high mortality. The antenatal diagnosis of CHD, by fetal echocardiography carries important advantages: 1) prenatal diagnosis is associated with less hypoxia, preoperative acidosis, fewer neurologic events, and early age to surgery [4]; 2) it provides opportunities for parental counseling; 3) it may optimize perinatal care in terms of changes in obstetric and neonatal management with multidisciplinary approach which should reduce mortality and morbility [5,6]. Routine neonatal examination fails to detect many affected babies, because a normal examination does not exclude serious cardiac malformation, including hypoplastic left heart syndrome or other pathologies such as interruption or coarctation of the aortic arch. Considering that about half of the babies with cardiac murmur, detected over the first few days of life, have a structural heart disease [7], these patients should be referred for early pediatric cardiological evaluation and definitive diagnostic echocardiography. Unrecognized neonatal cardiac malformation carries a serious risk of avoidable mortality, morbidity and handicap [8]. Regardless of this, all syndromic babies such as Down syndrome-affected babies who have a high prevalence of congenital heart disease, should be referred for early echocardiographic examination [1]. The key point is that echocardiography is an essential tool in the evaluation of neonates and has dramatically improved the accuracy of diagnosis of congenital heart disease.

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76.2 When and How to Treat – Preoperative Management of Neonates with CHD

The preoperative management of patients with critical congenital heart defects has a dramatic impact on overall outcome; it requires a coordinated, multidisciplinary approach with pediatric cardiologist, pediatric intensivist, surgeon, anesthesiologist, pediatric nurses, respiratory therapists, and family members. The major goal is to provide supportive care, clinical stabilization, and adequate cardiac output to ensure adequate tissue delivery. Advances in the surgical repair of congenital heart defects have allowed a trend toward a single stage repair. Nevertheless there are still two palliative procedures used for specific indications (Table 76.1): systemic-to-pulmonary artery shunt and pulmonary artery banding.

76.2.1 Systemic-to-Pulmonary Artery Shunt

The systemic-to-pulmonary artery shunt is a palliative procedure that is used to increase pulmonary blood flow in cardiac defects characterized by pulmonary outflow obstruction and clinical cyanosis. In these conditions, before surgery, the

Table 76.1 Indications for palliative procedure

1. Systemic-to-pulmonary arterial shunt
   - Right-sided obstructive lesion varying in severity from moderate pulmonary stenosis to pulmonary atresia
   - Tetralogy of Fallot
   - Single ventricle with pulmonary stenosis
   - Tricuspid atresia
   - Atrioventricular septal defect with severe pulmonary stenosis
2. Pulmonary artery banding
   - Tricuspid atresia with unrestrictive VSD
   - Single ventricle with unrestrictive pulmonary blood flow
3. Norwood procedure
   - Hypoplastic left heart syndrome

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

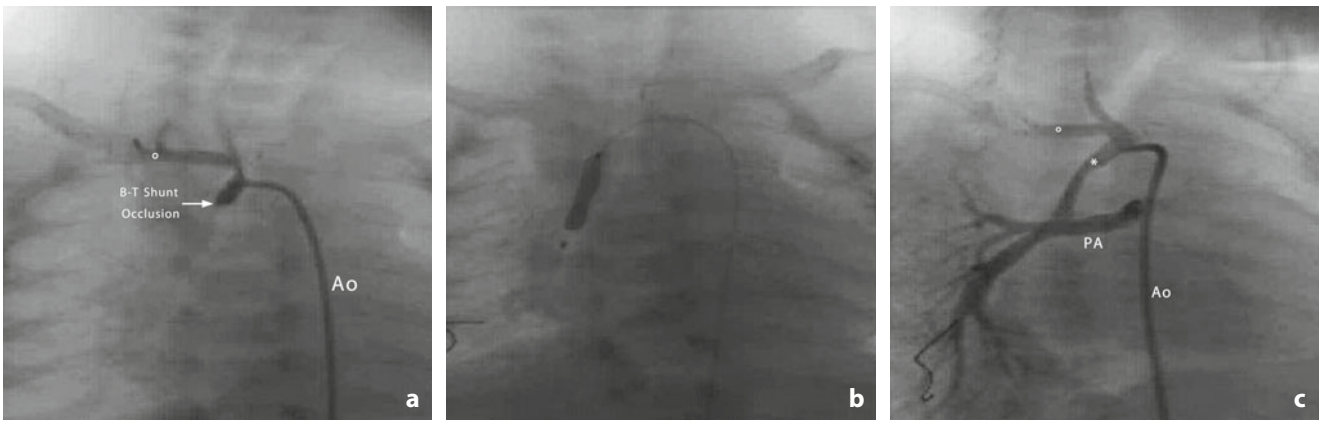

Fig. 76.1 **a** Injection in the right Blalock-Taussig shunt; the shunt is completely occluded. **b** Recanalization of the shunt with a balloon catheter. **c** Post balloon angioplasty: injection in the right modified Blalock-Taussig shunt; complete recanalization (*asterisk*). Right subclavian artery (*circle*), *AO* catheter in the aorta, *PA* pulmonary artery

ductus arteriosus is the main source of pulmonary blood flow; as the ductus closes spontaneously, severe hypoxemia, metabolic acidosis and death can occur. That is why prostaglandin (PGE_1) should be administrated by continuous intravenous infusion to maintain patency of the ductus arteriosus. In many cases supporting therapy is necessary to prevent vasoconstriction and avoid hypoxia, acidosis and hypercapnia which may increase pulmonary vascular resistance (PVR). It is important to maintain normal blood glucose, calcium levels and preservation of a neutral thermal environment [9]. The modified Blalock-Taussig shunt (MBTS) is the most commonly created systemic-pulmonary shunt in neonates [10]. This procedure consists of a polytetrafluoroethylene (Gore-Tex) graft placement between the innominate or subclavian artery and the ipsilateral pulmonary artery [11]; it aims at increasing pulmonary blood flow (PBF) and making pulmonary arteries grow in order to achieve a favorable anatomic condition for future correction. In particular situations such as Ebstein's Anomaly, pulmonary atresia with intact ventricular septum or hypoplastic left heart syndrome, transcatheter arterial duct recanalization and stenting can be considered as an alternative technique to a surgical shunt [12–14]. The postoperative management after Blalock-Taussig shunt insertion in neonates is generally uncomplicated. The saturation is about 75–80% in most of the cases. The extubation is deferred until pulmonary blood flow is adequate and the arterial saturation is stable. When the saturation is not adequate, following problems should be excluded: hypotension, shunt narrowing or occlusion, pulmonary edema and lung disease. If shunt narrowing or obstruction is detected, feasible treatment could be percutaneous balloon angioplasty (Fig. 76.1a,b,c), thrombolysis or surgery. Immediate postoperative anticoagulation with heparin is used, followed by daily aspirin therapy as long as the shunt is open.

Pulmonary edema can occur in cases of overcirculation, requiring if necessary reoperation to narrow the shunt size.

76.2.2 Pulmonary Artery Banding

Pulmonary artery banding is a palliative procedure performed in neonates affected by CHD associated with high pulmonary

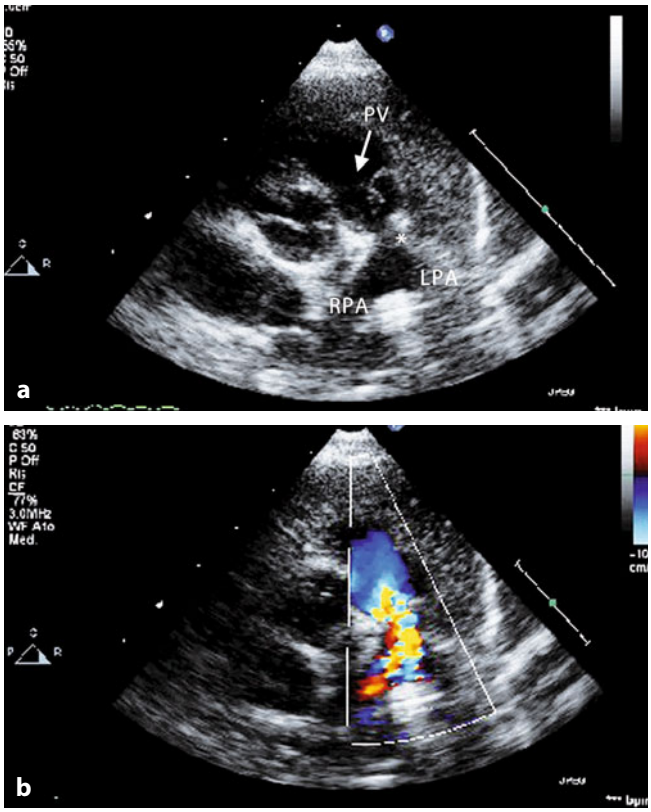

Fig. 76.2 Echocardiogram picture of pulmonary artery banding. **a** Pulmonary valve (PV); Pulmonary banding (*asterisk*). *LPA* left pulmonary artery, *RPA* right pulmonary artery. **b** Color flow picture showing flow acceleration across the banding

blood flow and CHF poorly controlled by medical therapy. The aim is to control the symptoms, to make the child grow properly and to prevent the development of pulmonary vascular disease.

The banding procedure consists of surrounding the main pulmonary artery with a synthetic band in order to create a stenosis limiting the pulmonary blood flow (Fig. 76.2) It can be performed either through sternotomy or anterolateral thoracotomy.

76.2.3 Norwood Procedure

The Norwood procedure is performed to treat the conditions that result in single ventricle circulation. Since in these conditions, the most urgent problem is that the heart is unable to pump blood to the systemic circulation, the object of the Norwood procedure is to connect the single ventricle to the systemic circulation. This procedure is prescribed in the treatment of hypoplastic left heart syndrome (HLHS), an association of anatomical anomalies involving the left side of the heart. HLHS includes varying degrees of hypoplasia of the left ventricle, critical stenosis or atresia of the mitral and/or aortic valve, along with some degree of hypoplasia in the aortic arch [4].

The surgical treatment for HLHS consists of three steps:

1. initial palliative reconstruction (STAGE I- Norwood procedure);
2. bidirectional Glenn shunt;
3. Fontan operation.

The first stage is performed to provide a systemic perfusion independent from the arterial duct, to preserve function to the single ventricle by minimizing excess pressure and volume work, and to allow normal maturation of the pulmonary vasculature. An atrial septectomy is performed to avoid obstruction of pulmonary venosus return and to allow blood flow from the left atrium (LA) to the right atrium (RA). The pulmonary artery and aorta are used to create the "neoaorta". Either a modified Blalock–Taussig (MBT) shunt or Sano shunt (right ventricle [RV] to main pulmonary artery [PA] conduit) is placed in order to supply pulmonary circulation [16]. The Sano shunt, compared with the BT shunt, results in a higher elevation of the diastolic blood pressure. Because the coronary arteries are perfused in diastole, the advantage expected in the Sano strategy is supposed to be a better coronary perfusion [17].

A new approach to the HLHS is known as "hybrid" procedure which consists of transcatheter stent implantation in the ductus arteriosus as well as surgical bilateral PA branches banding. The stent maintains systemic perfusion whereas the bands limit pulmonary blood flow [18, 19]. This procedure should be performed either in a catheterization laboratory or in a theatre where both angiographic as well as surgical capabilities and equipment should be available.

76.3 Pathologies which Require Correction in the Neonatal Period

76.3.1 Truncus Arteriosus

Complete mixing at the ventricular and arterial levels, with blood flow to the systemic and pulmonary circulation determined by pulmonary and systemic resistance is the consequence of truncus arteriosus. In the absence of pulmonary artery stenosis the normal fall in pulmonary vascular resistance (PVR) results in significant left-to right shunting, pulmonary overcirculation and symptoms of CHF. Due to the rapid development of pulmonary overcirculation, pulmonary hypertension and subsequent vascular obstructive, disease can occur as early as 3 months of life. Based on these data, the policy of many centers is to perform complete correction during the neonatal period [20]. The surgical repair consists of ventricular septal defect (VSD) patch closure, pulmonary artery removal from the common arterial trunk and RV to PA conduit implantation.

The combination of other cardiac malformations and truncus arteriosus, such as aortic arch interruption, outflow tract obstruction and multiple VSDs, carries high mortality with high risk of reintervention in survivors [21].

76.3.2 Transposition of the Great Arteries (TGA)

Most infants with TGA are diagnosed shortly after birth. Usually the babies need a balloon atrial septostomy (Fig. 76.3). Additional medical support is necessary to optimize clinical conditions.

The arterial switch operation (ASO) is the procedure of choice used to achieve complete physiological and anatomic correction. Current indications for ASO in newborns and infants must be taken into account in planning operative strategy, because the optimal time depends on anatomic features such as multiple VSDs or unbalanced ventricles. The key point is the morphologically left ventricular pressure at the time of presentation. Currently the optimal timing of ASO for babies with TGA without VSD varies from 5 to 15 days. Occasionally, newborns with TGA do not undergo early surgery because of later presentation, later diagnosis or both, or coexisting medical problems such as septic conditions or necrotising enterocolitis (NEC). In the ASO operation, the aorta and pulmonary artery are sectioned and their distal extremities are transposed and anastomosed; coronary arteries are then translocated to the neo aorta [22]. An alternative procedure is an atrial switch operation (Senning or Mustard operation) leaving the morphologically right ventricle in the systemic circuit. Despite the technical difficulties associated with the arterial switch operation and the problems related to postoperative management,

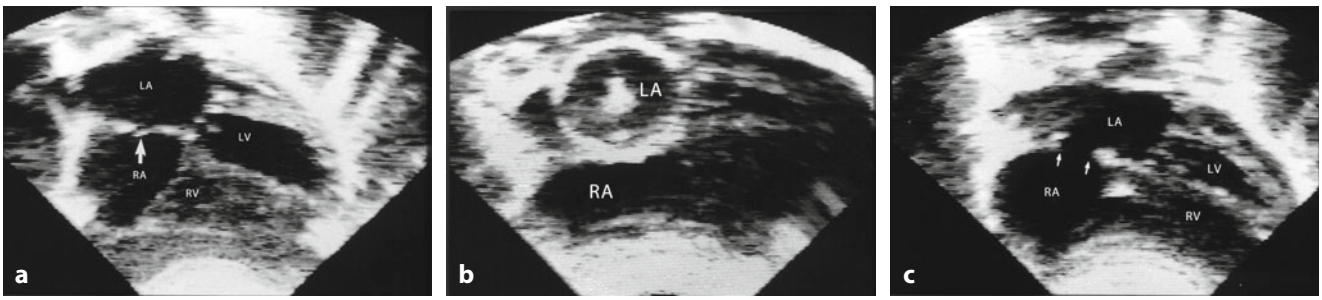

Fig. 76.3 Cross-sectional echocardiography from the subcostal approach demonstrating the septostomy procedure. **a** Short frame demonstrating the interatrial septum before the septostomy. **b** Catheter in the left atrium across the interatrial septum. **c** The interatrial communication (*arrows*) after balloon septostomy. *LA* left atrium, *RA* right atrium, *RV* right ventricle, *LV* left ventricle

early mortality is now similar to that of the atrial switch operation, whereas long-term results are better [23–25].

76.3.3 Total Anomalous Pulmonary Venous Return (TAPVR)

TAPVR is a congenital defect in which the pulmonary veins drain anomalously into a systemic venous structure (see Chapter 75). In obstructed TAPVR, correction is usually performed at the time of presentation, especially when the child is markedly cyanotic [26]. In unobstructed TAPVR, timing of correction varies with the severity of the clinical features: generally babies present with symptoms of CHF later in infancy, at that time correction should be carried out.

76.3.4 Ebstein's Anomaly

Hemodynamic abnormalities in this malformation are related to the severity of tricuspid regurgitation, the size of the functional RV, and the degree of the right-to left shunting across the atrial septal defect (ASD). Ebstein's anomaly can be associated with ASD and pulmonary outflow obstruction. If there is a severe tricuspid regurgitation in utero, massive cardiomegaly, secondary pulmonary hypoplasia, hydrops fetalis or a combination of these can occur [27]. The Ebstein's anomaly has a clinical spectrum that varies from severe cyanosis and circulatory collapse in the newborn to minimal or no symptoms in child or adult. Once the diagnosis is made, the newborn may require support with PGE_1 infusion and nitric oxide, and definitive surgical management is undertaken. For neonates, therapy is tailored to the severity of the malformation and the degree of RV outflow tract obstruction. Surgery may involve ligation of the duct, placement of a systemic to pulmonary shunt, creation of a functional tricuspid atresia or tricuspid valve repair [28]. Mortality is strongly related to severe tricuspid regurgitation and pulmonary hypoplasia.

76.3.5 Right-sided Obstructive Lesions

76.3.5.1 Tetralogy of Fallot (TOF)

There is a wide spectrum of anatomical variability in TOF with and without pulmonary atresia (TOF/PA). Clinical presentation depends on the severity of the anatomical defects, particularly on the degree of right ventricular outflow tract obstruction, the amount of right-to-left shunt through the ventricular septal defect and the size of pulmonary arteries, as noted in the previous section. Echocardiographic examination provides all the information required to plan surgical strategy (Figs. 76.4 and 76.5). Therefore cardiac catheterization is needed only when the diagnostic dilemma is still not sorted out like pulmonary or coronary artery anatomy, presence of aorto-pulmonary collateral arteries.

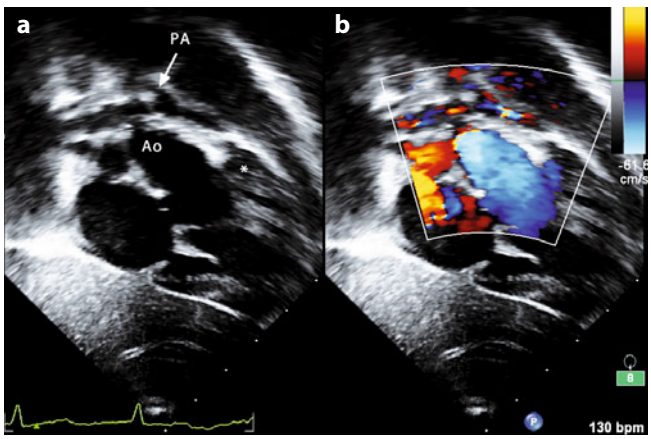

Fig. 76.4 **a** Right subcostal anterior oblique view in tetralogy of Fallot demonstrating the antero-cephalad deviation of the outlet septum into the right ventricular outflow tract. Infundibular pulmonary stenosis (*asterisk*). Significant hypoplasia of the pulmonary trunk and pulmonary arteries. **b** Color Doppler imaging showing turbulence and flow acceleration in the right ventricular outflow tract. The turbulence continues into the pulmonary trunk and pulmonary arteries. *AO* aorta, *PA* pulmonary artery

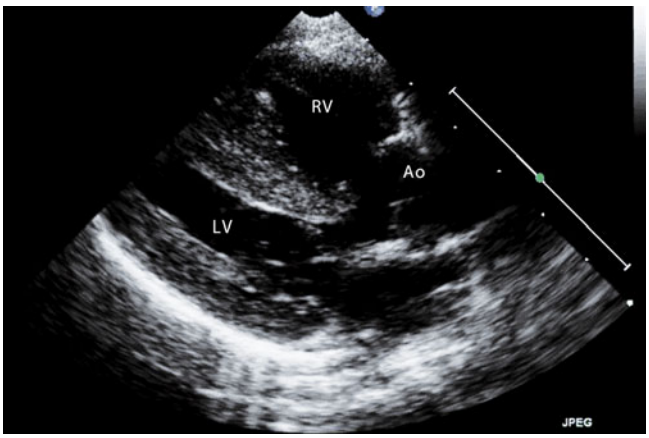

Fig. 76.5 Two-dimensional echocardiogram (modified parasternal long axis view) demonstrating large ventricular septal defect, aortic override and the right ventricular hypertrophy typical of tetralogy of Fallot

All patients with TOF and TOF/PA require surgical intervention. Initial medical management depends on clinical presentation which also depends on the severity of right ventricular outflow tract obstruction (RVOTO). When RVOTO is significant, neonates present with cyanosis, hypoxemia and metabolic acidosis: prostaglandin infusion should be started to maintain ductal patency and surgical palliation or repair after stabilization should be planned. Most commonly palliation consists of a subclavian artery-pulmonary shunt such as the modified Blalock-Taussig shunt, performed without cardio-pulmonary bypass. Due to the advances in neonatal cardiac surgery, some centers have been performing complete repair in neonatal period [29, 30]; major concerns remain regarding cardiopulmonary bypass complications, associated risk of longer stay in the intensive care unit, neurobehavioral abnormalities [30, 31], and anatomical issues. Other concerns include VSD patch closure, subpulmonary obstruction relief and pulmonary artery reconstruction through a transventricular approach [32, 33]. The ideal age for complete correction is still under discussion [29, 30, 34] although it is a common practice to proceed to complete correction within 4–6 months of age.

76.3.6 Pulmonary Valve Stenosis (PS)

PS is characterized by thickened, doming pulmonary valve leaflets with hypertrophied RV and normal tricuspid valve annulus. In critical PS, transcatheter balloon dilatation is the procedure of choice. The procedure is also recommended when RV pressure exceeds half the systemic pressure. Angiography and invasive pressure measurements during cardiac catheterization confirm the echocardiography diagnosis and allow measurement of the pulmonary valve which is fundamental to perform balloon valvuloplasty (Fig. 76.6). An oversized balloon, 1.2–1.4-fold the size of the annulus is used. Balloon valvuloplasty is effective and restenosis is uncommon [35]. In the presence of dysplastic valves such as in Noonan syndrome, valvuloplasty is less effective, requiring subsequently surgical valvotomy. Quite often, after critical PS balloon dilatation, cyanosis persists due to a persistent right to left shunt at the level of foramen ovale as a result of significant RV hypertrophy and diastolic dysfunction; in this situation an adequate pulmonary flow should be guaranteed either by keeping the arterial duct open with PGE_1 infusion or stent implantation or by performing a MBT shunt between the aorta and pulmonary artery.

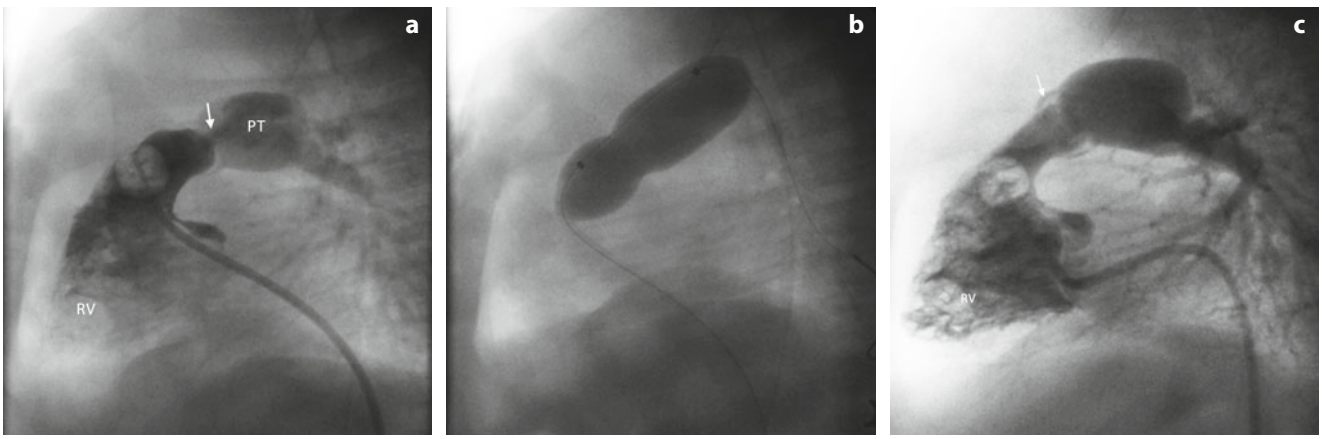

Fig. 76.6 A right ventriculogram in neonate with pulmonary valve stenosis. **a** A lateral view demonstrating systolic doming of the stenotic leaflets and narrow jet of contrast into PA (*arrow*). Right ventricle (RV); pulmonary artery (PA). **b** A guidewire is passing through the catheter from the right ventricle (RV). The balloon is inflated with diluted contrast until the balloon "waist" disappears. **c** A repeat right ventriculogram showing markedly improved antegrade flow through the stenotic pulmonary valve (*arrow*)

76.3.7 Pulmonary Atresia with Intact Ventricular Septum (PA/IVS)

PA/IVS represents a congenital heart defect with a wide spectrum of morphological heterogeneity. The morphologic aspects vary with respect to tricuspid valve size, RV hypoplasia, infundibular development and presence of coronary stenosis or coronary-RV cavity fistulas, called sinusoids. These connections are considered persistent communications which do not involute during intrauterine life due to the development of RV suprasystemic pressure; they might be found in up to 50% of the cases [36]. All the morphological aspects could be fully investigated by echocardiography and cardiac catheterization. Complete hemodynamic and angiographic study is essential even if a detailed diagnosis is carried out by echocardiography.

Transcatheter pulmonary valve radiofrequency perforation followed by pulmonary valve balloon dilatation is an effective procedure (Fig. 76.7) in order to decompress the right ventricular cavity and to reduce tricuspid valve regurgitation [38]. Subsequent postcatheterization management could be challenging because arterial duct stenting or MBT shunt insertion might be necessary in order to optimize the effective pulmonary blood flow.

Another alternative approach could be surgical pulmonary valvotomy with or without MBT shunt at the same time.

Babies with sinusoid-coronary artery dependent circulation should not undergo right ventricular decompression as this procedure could cause myocardial infarction due to an acute reduction of RV cavity pressure and consequently in the coronary artery perfusion pressure [37]. Therefore a palliative surgical approach (MBT shunt and atrial septostomy in case of restrictive ASD) could be a good option for these cases.

76.3.8 Left-sided Obstructive Lesions

76.3.8.1 Aortic Valve Stenosis (AS)

The treatment of AS depends on the degree of obstruction and the presence of systemic hypoperfusion. Neonates presenting with collapse or congestive heart failure need emergency treatment. PGE_1 infusion should be started to maintain ductal-dependent systemic flow. In many cases inotropic support is also required. The treatment of choice for neonatal congenital aortic stenosis is transcatheter balloon valvuloplasty (Fig. 76.8). Vascular access is usually retrograde using the carotid artery. However an antegrade approach using the

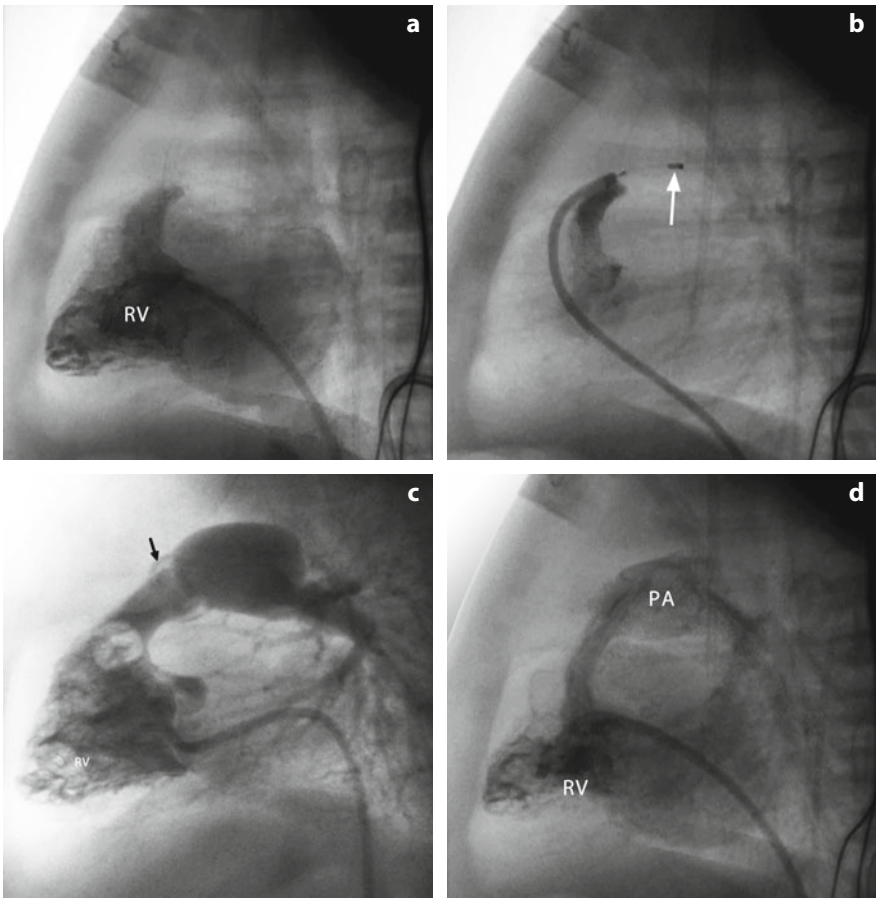

Fig. 76.7 Transcatheter intervention for pulmonary atresia. **a** Lateral view of a right ventricular (*RV*) injection in neonate with pulmonary atresia and intact ventricular septum. The RV outflow tract ends blindly at the level of the atretic pulmonary valve. No contrast crosses the pulmonary valve plate. **b** The lateral view angiography in the infundibulum: the tip of the radiofrequency perforation wire just positioned below the atretic pulmonary valve (*arrow*). **c** A balloon catheter submaximally inflated across the atretic valve. **d** Injection in the right ventricle (*RV*) demonstrating the continuity created between the RV and the pulmonary artery (*PA*)

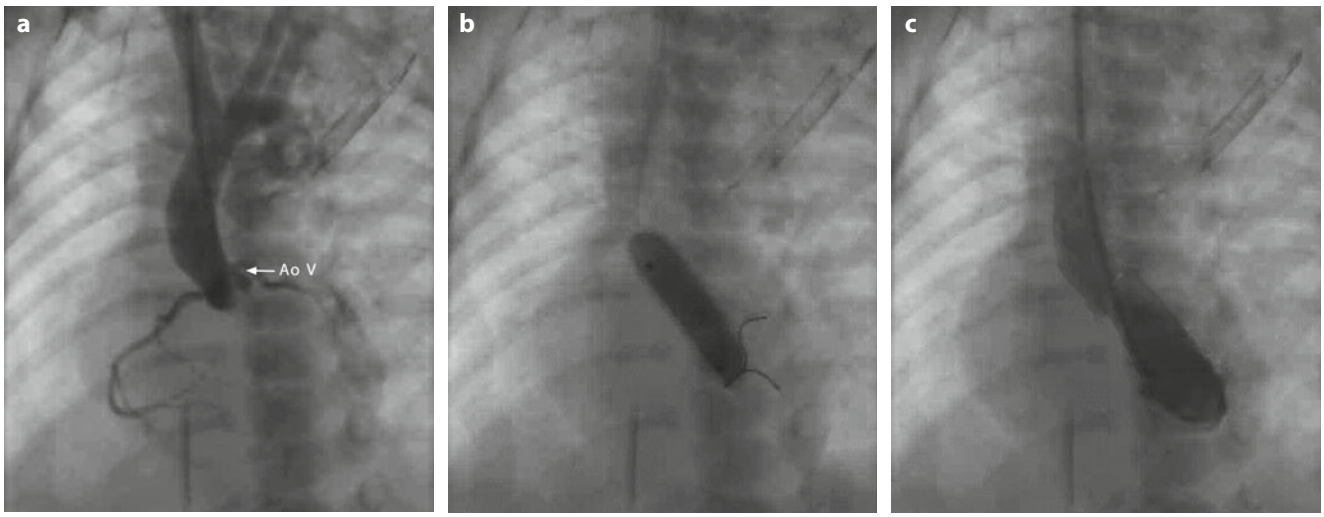

Fig. 76.8 **a** Carotid artery injection showing bicuspid aortic valve. **b** Balloon inflated across the aortic valve for valvuloplasty. **c** Injection in the left ventricle (*LV*) after valvuloplasty

umbilical or the femoral arteries has been described. The antegrade approach is associated with lower morbidity compared with the retrograde approach [39, 40]. Balloon valvuloplasty in neonates may be markedly effective; however limited long-term data have shown development of significant aortic valve regurgitation in up to 25% of patients treated with this technique [41].

76.3.8.2 Coartaction of the Aorta (CoAo)

The most common type of coarctation is located at the level of the isthmus, in the thoracic aorta, distal to the origin of the left subclavian artery. Neonates with CoAo develop symptoms once the duct closes. At that time systemic perfusion fails, and hypotension and acidosis occur. PGE_1 infusion should be given to keep ductal patency and supply systemic circulation as a palliative measure until surgical intervention is performed.

Treatment options vary from surgery to transcather intervention. Surgery with coarctation resection and end-to-end anastomosis or the subclavian artery flap technique is currently the first choice. However balloon angioplasty may be performed in circumstances where the infant is a poor candidate for surgery because of cardiovascular collapse [16]. Surgery for neonatal CoAo is associated with fewer reinterventions, improved aortic arch growth, no aortic aneurism formation, and a decreased need for antihypertensive medications when compared with neonates treated primarily with balloon angioplasty [42].

76.3.8.3 Interrupted Aortic Arch (IAA)

IAA consists of a disconnection between the ascending and descending aorta. Clinical presentation, preoperative management and timing of surgery are similar to critical CoAo. A VSD and patent ductus arteriosus are present in more than 90% of the cases [43]. VSD patch closure could be approached either during the same operation or in a second stage after having performed pulmonary artery banding at the same time as IAA repair.

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77

Arrhythmias and Heart Muscle Diseases

Fabio Mosca, Federico Schena and Anna Maria Colli

77.1 Arrhythmias

77.1.1 Introduction

Physiological cardiac electrical activity is characterized by sinus rhythm consisting of a regular sequence of activation of the atria (shown on the surface electrocardiograph [ECG] as a P wave), the atrioventricular (AV) node (PR interval), the ventricular myocardium (the QRS complex) and return to electrical rest (the T wave). The heart-beats show a regular cadence and the morphology of the EKG does not vary.

Arrhythmia is defined as the loss of regularity of sequence or morphology [1].

Arrhythmias are quite common in the neonatal period as both the regulatory systems and the neonatal myocardium are quite immature. Moreover the neonatal myocardium contains several bundles of potentially conducting tissue connecting the atrial to the ventricular myocardium, which may become surreptitiously active and allow rapid antegrade or retrograde conduction. On the other hand, conduction from the atria to the ventricles may be impaired and the regulatory interplay between the sympathetic and vagal mechanisms may produce variable responses compared to the adult [1, 2]. As ranges of normality for heart rate (HR) and ECG intervals differ at different age, Table 77.1 summarizes the normal ranges [1].

From a practical standpoint it is useful to approach arrhythmias based on both heart rate and ECG features. We therefore recognize tachyarrhythmias of atrial and ventricular origin and bradiarrhythmias which may originate form abnormally slow intrinsic pacemaker activity or be the consequence of a conduction defect at any level. Electrolyte disturbances, thyroid dysfunction and beta mimetic drugs all have an impact on cardiac rhythm and heart rate [1, 2].

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77.1.2 Ectopic Beats

Ectopic beats occur prematurely and may originate form the atrial (premature atrial contraction [PAC]) or ventricular myocardium (premature ventricular contraction [PVC]). They are extremely common in the fetus and newborn and their incidence in an otherwise healthy heart decreases with age.

PACs are characterized by a P wave occurring after an RR interval shorter than the previous one and a narrow QRS complex (Fig. 77.1). They originate from the right or left atrium or the proximal AV node. They are extremely common in the newborn population, are generally idiopathic and benign, and usually disappear within a few weeks. Occasionally, because the neonatal myocardium contains several bundles of conducting tissue connecting the atrial to the ventricular myocardium, a PAC reaching the ventricular myocardium may surreptitiously find one of these bundles in a conductive state and a short circuit ensues (reentry mechanism) generating a rapid sequence of beats known as supraventricular tachycardia (SVT). Occasionally, conduction through the ventricular myocardium does not follow the conduction bundles (aberrant conduction) and the ensuing QRS is wide resembling a PVC. Sometimes the PAC occurs very early and is not conducted beyond the atrial myocardium determining significant bradycardia.

PVCs are the most common arrhythmia in the pediatric age group and are usually benign if the heart is normal and the QT interval is normal for heart rate. They are characterized by an early and wide (> 80 msec) QRS complex not preceded by a P wave and followed by a compensatory pause, the sum of the preceding and subsequent RR intervals being equivalent to the sum of two sinus RR intervals. Occasionally they may occur regularly every other, third or fourth beat: in this case we speak of bi, tri and quadrigeminy. PVCs commonly occur in the presence of electrolyte imbalances, especially hypokalaemia.

Isolated PACs or PVCs require no treatment other than correction of electrolyte imbalances and withdrawal of cardioactive drugs (caffeine, theophylline, inotropes) unless they

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 77.1 Normal values of heart rate and ECG intervals according to age

Age	Heart Rate beats/min	PR interval	QRS interval	QTS axis (degrees)
Newborn	120–180	0.08–0.14 sec	<0.065 sec	+59 – +192 (+135)
1–12 months	120–180	0.08–0.14 sec	<0.080 sec	+31– +114 (+75)

QT interval requires correction for heart rate according to Bazett's Formula (QTc = measured QT (msec)/ √preceding RR interval (msec). Normal < 440 msec.

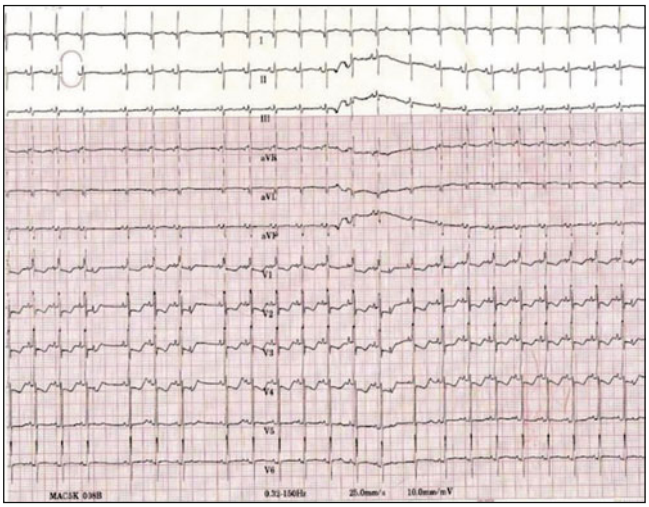

Fig. 77.1 ECG tracing showing supraventricular ectopic beats

are blocked and the ensuing heart rate is too slow or they occur in the context of long QT syndrome. When they occur during the vulnerable phase of myocardial electrical activity (the descending limb of the t wave) they may spark runs of ventricular tachycardia (VT) [1, 2].

77.1.3 Atrial Tachycardias

77.1.3.1 Sinus Tachycardia

Sinus tachycardia is actually not an arrhythmia in itself, the normal sinus activation occurs at a higher rate than normal for age. Always secondary, it is characterized by the variability of the RR interval as well as by the gradual onset and resolution. Treatment is based on the correction of the cause (e.g., fever, anemia, respiratory failure however slight, hyperthyroidism, sepsis, drug effect) [1, 2].

77.1.3.2 Supraventricular Tachycardia (SVT)

SVT is the most common atrial arrhythmia in the neonate with an incidence of 1–2:1000. It may occur in the absence of any myocardial disease and, amongst the congenital cardiac defects, the ones where it most commonly occurs are Ebstein's anomaly, congenitally corrected transposition of the great arteries, hypertrophic cardiomyopathy, tricuspid atresia and mitral valve prolapse (usually not a neonatal problem). It may complicate the long-term follow-up of extensive atrial surgery such as Mustard or Senning procedures for transposition of the great arteries.

SVT is characterized by a rapid heart rate (> 200/min). On the ECG it is characterized by narrow regular QRS complexes and the P wave is usually retrograde (it follows the QRS and is inscribed on the T wave) (Fig. 77.2). If the episode is long standing the repolarization may show myocardial suffering. SVT may be due to a reentry mechanism through an accessory pathway (commonly known as Wolff-Parkinson-White syndrome) with the circuit starting from the natural pathway and the return through the accessory pathway (orthdormic) or viceversa (antidromic) or sometimes through a permanent reentry in the AV node (permanent junctional reentry tachycardia [PJRT]).

Another mechanism is reentry in the absence of an accessory pathway (reciprocating reentry tachycardia and intra-atrial reentry) and the third possible mechanism is the presence of an abnormal automaticity with or without a specific focus (ectopic atrial tachycardia, polymorphic atrial tachycardia and junctional ectopic tachycardia).

Episodes of SVT may be short lived and completely asymptomatic (e.g., a casual finding on the monitor or ECG) or sustained, and if so they may lead to heart failure and shock especially if HR > 250/min for > 24–48 hours. In fact,

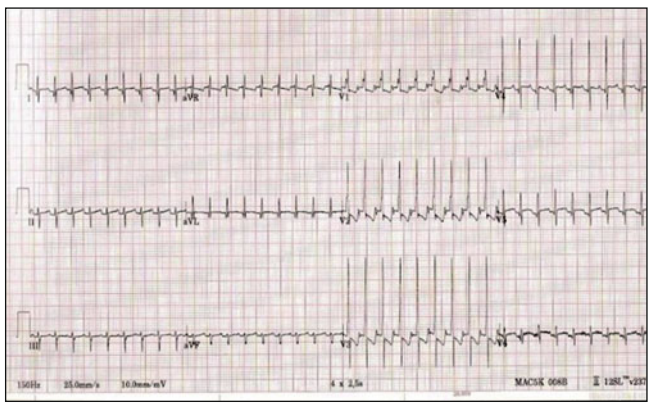

Fig. 77.2 ECG tracing of the same neonate during an episode of STV. Note the P waves inscribed on the T wave (retroconduction)

tachycardia occurs at the expense of diastole and this is the time when coronary flow to the myocardium occurs. Typically SVT has an abrupt onset sometimes prompted by a PAC and an equally abrupt end, distinguishing it from sinus tachycardia (the HR in the newborn may be similar) where both onset and resolution are gradual [1–5].

77.1.3.3 Atrial Flutter and Atrial Fibrillation

Atrial flutter (AFl) is characterized by rapid atrial reentry with atrial rates > 300/min. There is a variable degree of associated heart block so ventricular rate may be almost normal and occasionally, as the degree of block may vary in the same patient, irregular. It is rare, occurs more commonly in the fetus, is associated in most cases with a normal heart and the prognosis is good, recurrences being quite the exception.

Atrial fibrillation (AF) is virtually unknown in the newborn and is usually associated with either surgical scars or myocardial disease. Atrial activity is completely disorganized and ventricular rate is consequently irregularly irregular [1–5].

Treatment of SVT requires interruption of the reentry circuit. Unless it has been sustained and longstanding, when it may become refractory to treatment, SVT commonly responds to vagal maneuvers, the safest and most common of which consists of eliciting the diving reflex (apnea induced by stimulating the airways by placing a bag full of ice at melting temperature on the nose and mouth, just as if the baby was diving in cold water) or the rapid intravenous infusion of adenosine sulphate. Adenosine blocks the AV node and either interrupts the circuit or allows atrial flutter to become evident. If adenosine fails to interrupt the SVT permanently it is appropriate to use an antiarrhythmic such as flecainide or propafenone both as a bolus and as maintenance therapy. Digoxin, amiodarone and sotalol are also used alone or in combination with flecainide and propafenone. If the patient is hemodynamically unstable synchronized D/C shock with energy delivered at 0.5–4 Joules/kg is indicated. Ablation of abnormal pathways is not indicated in the newborn period and, once instituted, drug treatment must be continued for one year to prevent recurrences. Atrial flutter and fibrillation do not respond to vagal manoeuvres but require drugs or D/C shock depending on hemodynamics. Table 77.2 summarizes the dosages of the most common antiarrhythmic drugs used in pediatrics [2–15].

77.1.4 Ventricular Tachycardias

Ventricular tachycardias (VT) originate below the His bundle. They are characterized by a wide QRS complex on the ECG. Clinical presentation includes syncope and palpitation but it may be completely asymptomatic. VT are extremely uncommon in the newborn and when present are usually secondary to primary cardiac muscle diseases (including arrhythmogenic right ventricular dysplasia), ion channel

Table 77.2 The most common antiarrhythmic drugs and the currently accepted pediatric dosages

Drug	Attack dose (i.v.)	Maintenance dose i.v.	Maintenance dose per os
Adenosine	0.05–0.25 mg/kg Rapid bolus	N/A	N/A
Amiodarone	5mg/kg over 30’	10 mg/kg/day May be repeated	Attack dose p.o. 10 mg/kg/day for 10 days followed by 5 mg/kg/day
Atenolol	N/A	N/A	1mg/kg/day q.i.d. or b.i.d
Digoxin	20mcg/kg/day in 3 doses subdivided as ½ dose, ¼ dose + ¼ dose	1/3 of the attack dose (b.i.d)	Attack p.o. 30 mcg/kg/day in in 3 doses subdivided as ½ dose, ¼ dose + ¼ dose Maintenance 10 mcg/kg/day
Flecainide	1–2 mg/kg over 30’	2–6 mg/kg/day	2–7 mg/kg/day as b.i.d., t.i.d. or q.i.d.
Lidocaine	1 mg/kg as a single dose to be repeated every 5–10 minutes to effect up to max dose 3 mg/kg It may be given by endotracheal route at 1 mg/kg	20 + 50 mcg/kg/min	
Propafenone	0.3–1mg/kg over 30’ or 2 mg/kg over 2 hours	1–2mg/kg/day	10–15 mg/kg/day t.i.d.
Metoprolol	N/A	N/A	1 mg/kg/day q day or b.i.d.
Propranolol	0.01–0.1mg/kg/dose as a slow bolus	N/A	2–5 mg/kg/day t.i.d. or q.i.d.
Nadolol	N/A	N/A	1 mg/kg/day
Sotalol	N/A	N/A	4–9 mg/kg/day b.i.d. or t.i.d.
Verapamil	Not used in the first year of life because of risk of electro mechanical dissociation	N/A	2–6 mg/kg/day b.i.d. or t.i.d

Notes:
Flecainide and propafenone must be used in the hemodynamically stable patient and when systolic function is preserved.
Digoxin is usually considered a second choice and/or in association with another drug.
Amiodarone requires serial checks of thyroid function.
Lidocaine must be given cautiously in patients with low cardiac output. It is contraindicated in complete heart block and in wide complex tachycardia secondary to accessory conduction pathways.

diseases (e.g., Long QT syndrome: LQTS), cardiac tumors (e.g., rhabdomyomas), myocarditis, surgical scars or problems involving the coronaries (e.g., Kawasaki disease) electrolyte imbalances or drugs. The most common form in the newborn is the "torsade de pointe" or polymorphic VT, typically found in carriers of LQTS as a result of abrupt sympathetic stimulation or extreme bradycardia. LQTS is a genetic disorder of the ion channels of the myocardial membrane, causing electrical instability and malignant ventricular arrhythmias. The two best known variants are autosomal dominant (Romano-Ward, incidence of 1/5000–10,000 live births) and autosomal recessive, associated to deafness (Jervell-Lange-Nielsen). At a molecular level there are several genotypes identified, differing not only by the location of the responsible gene but also by clinical and EKG characteristics. The best known genotypes are:

- LQTS1 (K channels, gene located on chromosome 11) where VT occurs in response to stress or emotion. VT occurs frequently but mortality is low and response to beta blockade (metoprolol) excellent
- LQTS2 (K channels, gene located on chromosome 7) VT occurs in response to auditory stimulation. Mortality is high and treatment includes association of beta blockade and KCl supplements
- LQTS3 (Na channels, gene located on chromosome 3) VT occurs mostly during sleep or extreme bradycardia. Events are rare but mortality is high. Treatment includes implant of a pacemaker with defibrillator capabilities to counteract extreme bradycardia as well as arrhythmic events.

LQTS is one of the mechanisms underlying Sudden Infant Death Syndrome and the only one for which a simple screening method is available. In fact patients with LQTS fail to adjust QT length according to heart rate and this is detected by an abnormally long QT interval on the ECG. Finding a long QT on the ECG prompts not only evaluation of the variations over the 24 hours by Holter monitoring, but search of relevant mutations, especially if family history is positive [1, 2].

77.1.5 Bradyarrhythmias

Bradyarrhyhtmias considered physiological in the pediatric age group include:

- in the term baby: transient sinus bradycardia (HR 80–90 beats per minute) especially common after birth asphyxia;
- in the preterm baby sinus bradycardia associated with apnea;
- throughout childhood transient junctional rhythm and wandering pace maker, especially occurring during sleep.

Sustained bradycardias on the other hand are usually due to conduction disturbances such as sino-atrial block or various degrees of AV block.

Of these the so called 2nd degree block with Wenckebach phenomenon, in which the atrioventricular conduction time (measured by the PR interval) progressively lengthens until one P wave is not conducted at all, is physiological during sleep and requires no treatment.

The most common pathological bradycardia is complete AV Block (CHB), characterized on the ECG by independent atrial and ventricular activity. In the fetus and newborn it is usually congenital, either associated with congenital heart disease, especially corrected transposition of the great arteries, left isomerism or univentricular heart, or isolated.

Congenital CHB on the other hand shows ventricular rates well below normal and may lead to heart failure or sudden death. The most common cause of perinatal AV block is the presence of anti Rho antibodies in mother's blood, (which may well predate onset of full blown clinical picture of lupus), although other forms of myocarditis may affect the conduction tissue [1, 2].

77.2 Heart Muscle Diseases

77.2.1 Introduction

The term cardiomyopathy defines primary heart muscle disorders, where "primary" indicates that the changes are not due to congenital cardiac lesions, valvular diseases or hypertension. They are characterized by an often progressive systolic or diastolic dysfunction of the left ventricle, or both.

Classically we recognize three different forms: dilated, hypertrophic and restrictive, although the evolution from one to the other has been recognized and described. The most recent classifications also include arrhytmogenic right ventricle (RV) dysplasia (virtually unknown in the newborn for its degenerative nature) and non compaction of the left ventricular myocardium (NCLVM). The incidence of cardiomyopathies in the first year of life is 5–9/100,000 infants (much higher than the overall incidence in the pediatric group (1–2/100,000 per year before 18 years of age) [16, 17].

In a significant proportion of patients the onset of the clinical picture is at birth or within the first month of life. It is noteworthy that diagnostic criteria used by the major international registries exclude some secondary forms such as the cardiac hypertrophy in the infants of diabetic mothers (IDM), a common finding in the newborn.

Besides the different epidemiology, cardiomyopathies occurring in infants have a more severe prognosis. In fact they are mostly part of a more generalized metabolic, neuromuscular or genetic problem with multisystem involvement (determining the overall prognosis) compared with the later onset, primitive familial forms [18].

We shall discuss in detail hypertrophic and dilated cardiomyopathies, which are more common in newborns and infants, while we shall only briefly discuss NCLVM because its onset is usually well into infancy.

77.2.2 Etiology and Pathogenesis

77.2.2.1 Hypertrophic Cardiomyopathy (HCM)

Table 77.3 summarizes most pathologies associated with HCM. The most common syndromes associated with HCM are Noonan and Leopard (due to a defective PTPN11 gene) and Beckwith-Wiedemann, accounting for a large proportion of neonatal onset HCM [19].

Familial HCM is usually autosomal dominant and is due to a defect in one of the genes encoding for any one of the sarcomeric proteins. Most commonly the defect involves the myosin heavy chains, myosin binding protein 3, troponin I, tropomyosin and troponin T. Amongst these only the mutations of gene MHC7 may manifest themselves (in the heterozygous form) during the neonatal period while all the others usually appear beyond infancy. There are in the literature, however, cases of composite heterozygosis for two defects of the same gene or two different genes giving rise to extremely early onset and extremely severe forms.

The histological hallmark of HCM is myocardial fibres disarray associated with variable degrees of fibrosis. This picture is common to many of the cases occurring in the context of a syndrome or metabolic (e.g., mytochondrial) disorders.

In glycogen storage disorders such as pompe's, cardiomyocytes appear enlarged and show extensive sarcoplasmic vacuoles from lysosomal glycogen accumulation.

Macroscopically HCM is characterized by increased ventricular wall thickness, left ventricoular (LV) internal diameter may be normal or reduced and contractility is enhanced. Hypertrophy may involve either or both ventricles or involve only the interventricular septum asymmetrically. Septal hypertrophy may cause LV outflow tract obstruction (hypertrophic obstructive cardiomyopathy, HOCM) which is in turn enhanced by the systolic anterior motion (SAM) of the mitral valve. Also hypertrophy may be so massive as to reduce the LV to a virtual chamber and interphere with LV filling (diastolic dysfunction).

The unfavorable fiber/capillary ratio in severe hypertophy may cause myocardial ischemia and arrhythmias are the next most common complication.

Table 77.3 Etiology of hypertrophic cardiomyopathy in newborns and infants

Malformation syndromes
Noonan syndrome
LEOPARD syndrome
Beckwith-Wiedemann syndrome
Costello syndrome
Inborn errors of metabolism
Glycogen storage disease
Pompe disease (glycogen storage disease type II)
Glycogen storage disease with normal acid maltase
Glycogen storage disease type IX
Cori disease (glycogen storage disease type III)*
Danon disease
Mucopolysaccharidoses
Hurler syndrome*
Hunter syndrome*
Morquio syndrome*
Mythocondrial diseases and respiratory chain deficiencies
Leigh disease*
Complex I deficiency
Combined respiratory chain deficiencies
MELAS syndrome*
Barth syndrome
Sengers syndrome
Other oxidative phosphorylation disorder
Fatty acid oxidation and carnitine disorders
Primary or systemic carnitine deficiency
Carnitine palmitoyl transferase type II deficiency
Very long chain acyl-CoA dehydrogenase deficiency
Long chain acyl-CoA dehydrogenase deficiency
Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency
Multiple acyl-COA dehydrogenase deficiency
Congenital disorders of glycosylation
CDG type Ia
Neuromuscular disorders
Myotonic dystrophy
Nemaline myopahty
Other congenital myopathies
Familial isolated cardiomyopathy
Familial HCM with autosomal dominant inheritance
X-linked isolated cardiomyopathy
Familial HCM autosomal recessive inheritance
Familial HCM with Wolff-Parkinson-White syndrome

* Rarely seen in the newborn period.

77.2.2.2 Dilated Cardiomyopathy (DCM)

Forty percent of cases of DCM occurring in infancy present in the first year of life. In the majority of cases etiology remains unknown and we speak of idiopathic DCM. DCM as a sequela of viral myocarditis represents approximately 10–15% of cases, much rarer than in later ages and the same is true for DCM related to neuromuscular disorders. Another 10% of cases occur as familial, syndromic (e.g., Alstom's syndrome) or secondary to metabolic disorders (carnitine deficit, Barth syndrome).

In familial forms the autosomic dominant modality of transmission is prevalent; the majority of genes identified thus far encode for sarcomeric or cytoskeletal proteins, responsible for generating contractile force or transmitting mechanical impulses between adjacent cells [19].

Macroscopically the mechanical dysfunction at cellular level leads to reduced LV contractility, reduced LV ejection fraction and progressive LV dilatation. LV wall thickness may be reduced or increased and secondary AV valve regurgitation leads to atrial dilatation with its incumbent risk for atrial arrhythmias and thromboembolism.

77.2.3 Clinical Aspects

77.2.3.1 Hypertrophic Cardiomyopathy

In the forms with early onset, myocardial hypertrophy is present since fetal life and may be diagnosed in the course of a routine obstetric ultrasound. After birth, symptoms are generally due to diastolic dysfunction and include dyspnea, sometimes cyanosis (mostly acrocyanosis), feeding difficulties and failure to thrive. Physical examination may show a variably harsh ejection systolic murmur if LV outflow tract obstruction is present. Peripheral pulses are usually normal. Sometimes the diagnosis is incidental in a completely asymptomatic baby being investigated for suspected syndrome or metabolic disorder or because of family history of cardiomyopathy. Rarely, sudden death is the first manifestation of the disease. ECG shows LV hypertrophy (represented by high voltages in the left or all precordial leads), LV overload (ST–T changes), atrial hypertrophy and conduction disturbances. Chest X ray is relatively aspecific but may show cardiomegaly and pulmonary venous congestion. Two dimensional (2D) echocardiography establishes the diagnosis. Diagnostic criteria include increased thickness of the interventricular septum and the posterior wall in diastole (> 2SD for age or BSA), increased LV fractional shortening (FS), evidence of LV outflow tract obstruction and SAM of the mitral valve [19].

77.2.3.2 Dilated Cardiomyopathy

Presenting symptoms of DCM in the newborn are essentially those of cardiac failure and low output: tachypnea or dyspnea, sweating, tachycardia, pallor and poor peripheral perfusion, feeding difficulties, vomiting.

Cardiac auscultation is characterized by gallop rhythm and a systolic murmur from mitral regurgitation, peripheral pulses may be weak and the liver is often enlarged. Exceptionally the first alarm is an episode of acute life threatening event (ALTE). In a minority of cases diagnosis is made before the onset of symptoms in the course of evaluation for positive family history or prenatal diagnosis of multiple malformations (syndromic picture) or metabolic disorders.

Chest X-ray shows cardiomegaly and pulmonary venous congestion which may reach pulmonary edema, occasionally the left atrium may be so enlarged as to cause left lower lobe atelectasis.

ECG findings are often aspecific and may show sinus tachycardia, flat or inverted T waves in the left precordial leads or left atrial enlargement, and, as the disease progresses, ventricular arrhythmias, especially ventricular tachycardia or polymorphic ventricular tachycardia (torsade de pointe), may occur. Diagnosis is easily established by echocardiography which shows increased LV diastolic diameter, reduced systolic function indices (shortening and ejection fractions). Wall thickness may be normal or reduced and there may be intracavitary thrombus. Color doppler may show mitral regurgitation [20].

77.2.4 Differential Diagnosis

77.2.4.1 Hypertrophic Cardiomyopathy

Left ventricular hypertrophy secondary to poorly controlled maternal diabetes is a relatively common finding in infants of diabetic mothers (IDM), cardiac dysfunction may be severe and mimic primitive HCM, but natural history is good with gradual resolution of the morphologic and clinical picture within the first few weeks of life. Cardiac hypertrophy secondary to hypertension (e.g., from renal disease) and left ventricular outflow tract obstruction (especially coarctation of the aorta). Severe RV hypertrophy may be caused by premature ductal constriction which may be the consequence of maternal ingestion of non steroidal anti inflammatory drugs in the latter weeks of pregnancy. In addition myocardial hypertrophy in the premature baby may be secondary to steroids (for chronic lung disease) or long-term inotropic drugs administration. Correct differential diagnosis is paramount, as the latter forms are usually reversible once the drug is withdrawn.

Once a primary cause for myocardial hypertrophy has been ruled out, the finding of HCM in a newborn mandates an accurate and specific family history to establish whether there is a recurrence of HCM, accurate search for even subtle physical findings suggestive of a genetic (syndromic) picture and a complex workup for inborn errors of metabolism which must include blood and urinary screening for amino or ketoacids and even muscle or skin biopsy for study of mitochondrial DNA and search for specific histological picture of certain myopathies [21].

77.2.4.2 Dilated Cardiomyopathy

Ventricular dilatation with associated clinical signs of cardiac failure may be the consequence of congenital viral infections as well as of severe chronic fetal anemia and both mimic DCM. Specific IgG and IgM and hemoglobin levels are usually diagnostic in those cases. Amongst structural congenital anomalies CMD may be secondary to severe chronic myocardial ischemia secondary to anomalous origin of the left coronary artery from the pulmonary artery, in which the neonatal myocardium receives deoxygenated blood. Therefore when approaching a newborn or an infant the origins of the coronary arteries from the ascending aorta must be clearly established as the origin of a coronary artery from the pulmonary artery is difficult to be univocally established by 2D echo, but surgery may allow a normal left ventricular function to be reestablished.

Neonatal HIV infection may determine LV dysfunction in children but rarely in infants.

The finding of DCM, just like HCM, requires an accurate and specific family history inquiring for cases of inheritable metabolic or neuromuscular disorders. Timely detection of carnitine deficiency must be actively sought as the clinical picture is often curative. On the other hand post–myocarditis DCM may not be easily diagnosed on serology and endomyocardial biopsy may be required [21, 22].

77.2.5 Prognosis

77.2.5.1 Hypertrophic Cardiomyopathy

Prognosis of HCM diagnosed in the first year of life is much worse than that diagnosed later on: in fact mortality reaches 25–30% in most published series. For those infants who survive the first year of life, the per year mortality rate slowly drops and realigns itself with that of cases diagnosed later in life. Cases diagnosed in utero or at birth appear to have an even higher mortality, although specific epidemiologic data are missing.

Prognosis is heavily influenced by the underlying cause with a one year survival of 30% in inborn errors of metabolism, 65–70% when a syndrome is present and 85% in idiopathic HCM [19].

77.2.5.2 Dilated Cardiomyopathy

Prognosis of DCM is variable but overall severe. Forty to forty-five percent of patients die or are transplanted after an average of 3 months after diagnosis.

Negative prognostic factors are presence of heart failure at onset, lowest LV ejection or shortening fraction. Post-myocarditis DCM has a slightly better prognosis while the onset secondary to inborn errors of metabolism has the worst. Improved survival in recent years is mostly due to the increasing number of patients reaching cardiac transplantation [20, 23].

77.2.6 Therapy and Treatments

77.2.6.1 Hypertrophic Cardiomyopathy

Very few cases (thus far only patients with pompe's disease) benefit from enzymatic replacement treatment; in all cases in which carnitine deficiency or other derangements of beta oxidation are suspected it is recommendable that supplementary carnitine be administered.

Heart failure treatment is based mostly on ACE inhibitors and diuretics; cases with LVOTO and diastolic dysfunction are treated with beta blockers (propranolol or atenolol) and in some cases with Ca antagonists (verapamil) despite it being generally held as contraindicated in the newborn. In a recent series of pediatric patients, administration of high dose beta blockers (propranolol 5–20 mg/kg/day) was associated with improved survival [24]. Inotropes are usually contraindicated as they enhance the degree of obstruction. Occasional cases of asymmetric septal hypertrophy are suitable candidates for surgical resection of septal hypertrophy. When there is no or no longer responsiveness to medical treatment, cardiac transplantation is indicated.

77.2.6.2 Dilated Cardiomyopathy

Treatment is based on common antifailure drugs (diuretics, ACE inhibitors, digoxin, inotropes). Many patients require additional antiarrhythmic drugs and anticoagulant or antiaggregant treatment; just like HCM, some cases associated with metabolic disorders respond well to treatment with carnitine at pharmachological dosages [23].

Ventricular assist devices, pacemakers for resynchronizing ventricular contraction, automatic implantable cardioverters, intraaortic balloon or extracorporeal membrane oxygenation have all been used as a bridge to transplantation though not in the newborn period.

Cardiomyopathies represent the indication to cardiac transplantation in 35% of cases in the first year of life and > 70% in older ages.

Current average survival after pediatric cardiac transplantation is approx 18 years, though some centers are investigating genetic therapy and stem cell treatment in some forms of cardiomyopathy.

77.2.7 Non-Compaction of the Left Ventricular Myocardium (NCLVM)

NCLVM is a recently identified form of cardiomyopathy characterized by the presence of deep muscular trabeculations in the LV wall establishing the presence of deep sinuses that determine the sponge-like appearance of the myocardium. Fifteen to eighteen percent of cases are familial although the specific genetic basis is far from established and is likely quite composite [25]. The gene encoding for tafazzin (responsible for Barth syndrome) has been involved in some cases and in others the cardiac picture was associated with dysmorphic features. The majority of pediatric cases are diagnosed in the first year of life, representing almost 10% of pediatric cases with an estimated incidence of 0.12 cases per 100,000 children, clinically NCLVM is characterized by variable degrees of systolic and diastolic LV dysfunction. Arrhythmias may also occur as well as intracavitary thrombi. Symptoms at onset include tachypnea, cyanosis, failure to thrive or weight loss, syncope or thromboembolic events. Chest X-ray may show cardiomegaly, ECG high ventricular voltages and ST-T wave changes.

Diagnosis is established by 2D echo and requires the following criteria: multiple LV wall trabeculations and multiple

deep recesses between trabeculae in continuity with the LV cavity usually visible by color Doppler, mostly at the level of the apex or mid ventricle. LV myocardium shows a two layered structure with an uncompacted/compacted ratio > 2 (or 1.4 in infants according to some authors). In some cases this evolves into echo pictures almost undistinguishable from either DCM or HCM. In selected cases magnetic resonance imaging (MRI) may be useful if echo is not diagnostic. Prognosis is very variable but may be serious with death or severe disability leading to transplantation as early as one year after diagnosis. Some forms show an undulating course with initial improvement and subsequent deterioration even years later.

Treatment is based on anti failure drugs, beta blockers for hyperkinetic arrhythmias and thromboembolic prophylaxis (acetylsalicylic acid) which is advised in all cases.

Some patients have benefitted form carnitine, coenzyme Q, thiamine and riboflavine supplementation, while others have been sent to cardiac transplantation [26].

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78

Blood Pressure Disorders in the Neonate: Hypotension and Hypertension

Jonathan M. Fanaroff and Avroy A. Fanaroff

78.1 Introduction

Blood pressure (BP) disorders are a common problem in neonates admitted to the neonatal intensive care unit (NICU). Additionally, there is an association between systemic hypotension and neonatal morbidities such as intraventricular hemorrhage [1]. Hypertension, while less common, can also lead to significant short and long-term morbidities. There remains, however, no standard definition of hypotension or hypertension in the neonatal period. Indeed, there is convincing evidence that wide variations exist among NICUs in both practice styles and approaches to blood pressure disorders. Barrington notes "The treatment of shock and hypotension in the preterm infant may be the area of neonatology where there is the greatest "intervention/data imbalance"; more babies receive more treatments with less supportive evidence than in virtually any other domain" [2].

In this chapter the current status of hypotension and hypertension in the neonatal period is reviewed. First we examine the measurement of neonatal blood pressure and then review the definitions of blood pressure disorders. Subsequently the causes, consequences and treatment of neonatal hypotension are presented. Finally the causes, clinical features, diagnostic studies and an approach to clinical management of neonatal hypertension are discussed.

78.2 Measurement of Neonatal Blood Pressure

The technical goal for measurement of blood pressure would be a method that is simple, reliable, non-invasive, painless, and gives a continuous measurement. Unfortunately this goal has not yet been obtained. Direct invasive measurement (via an arterial or peripheral artery catheter) is currently considered the optimum method. Usually, mean blood pressure rather than systolic pressure is used when judging the normality of data obtained from the indwelling arterial line because it is thought to be free of the artifact caused by resonance, thrombi, and air bubbles, but this may not always be true. The downside of invasive measurement relate to the risks of an indwelling catheter, such as thrombus formation, hemorrhage, or infection. Noninvasive indirect measurement includes oscillometric and automated Doppler techniques. Provided that cuff size is standardized, oscillometric measurement seems to be accurate within the normal range. However, concern has been expressed that, at the lower levels, it consistently overestimates the blood pressure providing false reassurance.

78.3 Definition of Hypotension and Hypertension

While many studies have looked at blood pressure ranges in neonates, there remains no standard definition of truly pathologic hypotension and hypertension. Fig. 78.1 shows data obtained in a study of 608 newborns admitted to Philadelphia area NICUs during the first 99 days of life [3]. One way to define abnormal blood pressure is based on normative values. Hence hypertension is often defined as sustained systolic (SBP) and/or diastolic (DBP) blood pressure more than two standard deviations above the mean values (≥ 95%) [4]. However, blood pressure varies significantly, in a linear fashion, with gestational age and birth weight. Gender may also be significant on the first day of life when extremely low birth weight (ELBW) (< 1000 g) males have lower blood pressure than females [5]. Blood pressure also increases daily during the week of life. Hence the definition of hypotension in the newborn has remained elusive and treatment algorithms are

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

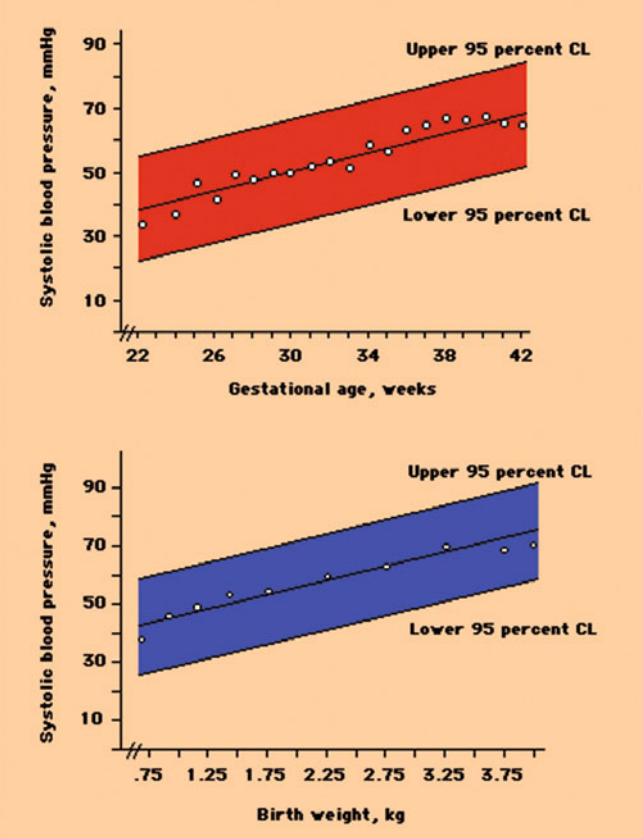

Fig. 78.1 Neonatal blood pressure. Linear regression of mean SBP on gestational age and birth weight in 329 infants admitted to NICU on day 1 of life. The mean SBP and 95 percent confidence limit (CL) for incremental gestational age and birth weight are plotted about the respective regression lines. Reproduced with permission from Zubrow AB, Hulman S, Kushner H, Falkner B. Determinants of blood pressure in infants admitted to neonatal intensive care units: a prospective multicenter study. Philadelphia Neonatal Blood Pressure Study Group. J Perinatol 1995; 15:470–479. Copyright 1995 Nature Publishing Group

based upon statistically defined gestational age-dependent normative blood pressure values and clinical judgment.

The dilemma remains when to treat and how. The traditional "two standard deviations below the norm" can be applied to the term neonate, but for preterm infants "normal for age" is a challenge. Furthermore, while almost a quarter century has elapsed since Versmold published his classic paper on blood pressure in the smallest premature infants, there is particular controversy over when to treat abnormal blood pressures in ELBW infants [6]. Whereas in adults and children normative data for systolic, diastolic and mean blood pressure is available, in infants most data sets have used "mean blood pressure" to define hypotension and determine when to intervene. Empirically the gestational age in weeks has been used to define the lower limits of mean blood pressure during the first day of life. But this is unreliable and the blood pressure should be looked at in the context of the clinical condition. Normal blood pressure in newborns varies with gestational age, birth weight and postnatal age, increasing by 1–2 mmHg per week for the first month. Blood pressure rises significantly during the first 72 hours of life irrespective of gestational age so that all preterm infants should have a mean pressure above 30 mmHg by this time [7].

Oxygen delivery to the tissues is influenced by cardiac output and blood flow more so than blood pressure, and, hence, values of blood pressure that are statistically abnormal are not necessarily pathologic. Shock is a complex clinical syndrome caused by acute circulatory failure. Hypotension (i.e., lower than expected blood pressure) frequently, but not always, accompanies shock. Shock is characterized (and defined) by inadequate tissue and organ perfusion, which may involve a single organ or all organ systems. Impaired perfusion not only results in inadequate delivery of oxygen and nutrients but also inadequate removal of metabolic waste products. Cellular function is disrupted and eventually there is cell death. Few data are available on the relation between blood pressure and systemic flow, cardiac output and neonatal morbidity and mortality but shock remains a major cause of neonatal morbidity and mortality [7]. In most very low birth weight (VLBW) neonates, cerebral blood flow autoregulation is indeed lost when blood pressure reaches the fifth percentile [8].

The incidence of hypotension is relatively high, especially in ELBW infants. Between 16 and 98% of extremely preterm infants receive treatment for hypotension in the first few days of life. This enormous variation has arisen because of a lack of reliable information to create an evidence base for intervention [9–11]. There is a significant variation among NICUs in the incidence, diagnosis, and approach to hypotension.

Al-Aweel et al [12] in a multi-center report found an almost three-fold variation among sites in the odds of hypotension [12]. Even more concerning, two of the six sites had a 5- to 30-fold higher use of vasopressors than other sites after risk adjusting for the occurrence of hypotension [12].

78.4 Clinical Features

In addition to low blood pressure the clinical features of hypotension in the neonate include varying combinations of tachycardia, bradycardia, and tachypnea, mottling of the skin, prolonged capillary refill time, cool extremities, and decreased urine output. A difference between the central and peripheral temperature may be indicative of hypovolemia or sepsis. Volume expansion will rapidly reduce the differential between central and peripheral temperature.

78.5 Causes of Hypotension

There are many causes of neonatal hypotension and shock syndromes. Therapy must be specifically directed at the

Table 78.1 Causes of neonatal shock

A. *Hypovolemia*
- a. Placental hemorrhage;abruption placenta previa
- b. Feto-maternal hemorrhage
- c. Twin to twin transfusion
- d. Birth trauma – subaponeurotic bleed
- e. Ruptured liver/spleen
- f. Massive pulmonary hemorrhage
- g. Disseminated intravascular coagulopathy
- h. Third space losses – Necrotizing entero-colitis

B. *Cardiogenic shock*
- a. Asphyxia
- b. Arrhythmias
- c. Congenital heart disease
  - i. ductal dependent lesions when ductus closes
  - ii. total anomalous pulmonary venous drainage
- d. Cardiomyopathy
- e. Myocarditis
- f. Air leak syndromes
  - i. Pneumothorax
  - ii. Inadvertent positive end expiratory pressure (PEEP)

C. *Sepsis and septic shock*

D. *Endocrine*
- a. adrenal hemorrhage
- b. adreno-genital syndrome

E. *Drug-induced hypotension*

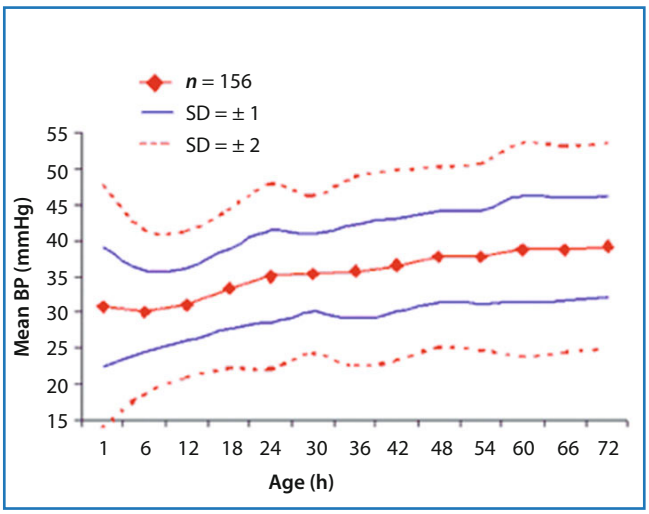

Fig. 78.2 Mean blood pressure of all ELBW infants in the first 72 hours of life

underlying cause. Table 78.1 lists the various causes of hypotension. Note that there are many other causes than hypovolemia, and, indeed in ELBW infants hypovolemia is relatively uncommon compared to other causes.

Cardiac output is the product of heart rate and stroke volume. Because of a limited ability to increase stroke volume, neonatal cardiac output is more dependent upon heart rate. Hence prolonged tachycardia or bradycardia compromise cardiac output. Stroke volume is dependent upon preload (ventricular filling), after load (systemic and pulmonary vascular resistance), and myocardial contractility. The treatment of hypotension is based upon manipulation of these parameters [9].

78.6 Significance of Blood Pressure Requiring Treatment in ELBW Infants

To explore the relationship between short and long term morbidity and neonatal blood pressure Fanaroff et al [7] performed a chart review of the 156 infants with birth weight between 401 and 1000 grams who were born during the period 1998–1999 and admitted to the NICU at Rainbow Babies & Children's Hospital. Blood pressure, obtained predominantly (81%) by umbilical arterial catheter (or by oscillometer 19%) was recorded hourly for the first 24 hours and then every 6 hours until 72 hours of life. Infants were then divided into two groups – those who received no treatment to support blood pressure (n = 97) and those who were treated for hypotension (n = 59). Infants receiving treatment weighed less at birth and were a week less mature than those who received no blood pressure support. They were also less likely to have been exposed to antenatal corticosteroids. Patients were considered to have treated hypotension if they received fluid pushes and/or pressors (dopamine and/or dobutamine) during the first 72 hours of life to elevate blood pressure. The infants underwent neurologic, developmental and formal audiologic testing. Fig. 78.2 shows the plot of the mean blood pressure, as well as one and two standard deviations above and below the mean during the first week of life. Overall there is a spontaneous increase in mean arterial pressure (MAP) from 32 at age 1 hour to 41 mmHg by the end of the first week of life. Infants requiring blood pressure support were more likely to have severe intracranial bleeds and had a higher mortality rate. Logistic regression analyses controlling for maternal socioeconomic status and coexisting neonatal morbidity was performed and at follow-up treated hypotension was significantly associated with delayed motor development (β: −6.0, SE: 3.1) and hearing loss (OR 8.9, CI 0.92-86.3) [7].

78.7 Treatment

Successful management of neonatal hypotension and shock mandates understanding the etiology and patho-physiology of the cardiovascular compromise. The ability to establish the etiology of the condition (hypovolemia, myocardial dysfunction or abnormal vasoregulation) at the bedside is limited. Management, despite efforts to use all the available evidence, often remains empiric. Volume replacement (fluid boluses) vasoactive medications and corticosteroids in various

combinations are the foundations of therapy. It is important not to be treating a number, but to consider the blood pressure in the context of the infant's total clinical condition. Frequent evaluation of vital signs, blood pressure, perfusion, urine output and neurologic status are used to guide the interventions. Although many hypotensive babies are hypovolemic, there are many other causes for hypotension and they should all be considered, including defining the hemodynamics echocardiographically, before pushing excessive volume.

The optimal means of restoring normal circulating blood volume, blood pressure and cardiac output have yet to be determined. Whether crystalloids or colloids are preferable is also unclear in newborns. In their Cochrane review, Osborn and Evans commented: "There is no evidence from randomized trials to support the routine use of early volume expansion in very preterm infants without cardiovascular compromise. There is insufficient evidence to determine whether infants with cardiovascular compromise benefit from volume expansion. There is insufficient evidence to determine what type of volume expansion should be used in preterm infants (if at all) or for the use of early red cell transfusions" [13].

Normal saline has been shown to be equally effective to albumin in restoring blood pressure in hypotensive preterm infants. Because it is efficacious, safer, readily available and inexpensive normal saline has become the crystalloid of choice for volume expansion [14]. Furthermore, in a follow-up of a randomized trial comparing crystalloid and colloid Greenough reported that 19/131 survivors had abnormal neuro-developmental status [15]. Although these infants differed from the normal group in birth weight, magnitude of colloid infusion received and oxygen exposure in addition to intraventricular hemorrhage, periventricular leukomalacia and postnatal corticosteroid exposure, regression analysis demonstrated that only colloid infusion related significantly to abnormal neuro-developmental outcome independent of other variables. Thus colloid infusions should be used with caution in the perinatal period and normal saline is the preferred fluid for volume repletion.

Although volume expansion is liberally used in newborn intensive care, little is known about its effects on hemodynamics or outcomes. Ewer et al [16], using data that were obtained from anonymized regional case notes of Project 27/28, a British National case-controlled study run by the Confidential Enquiry into Stillbirths and Deaths in Infancy, reported that newborns who received 30 mL/kg volume expansion in the first 48 h of life were more likely to die than those who received <30 mL/kg (OR 4.5, 95% CI 1.2, 17.2). More is not necessarily better!

Mayock and Gleason [17], by studying the effects of volume expansion with and without hypoxia in immature lambs provide some insight into this problem. They note that rapid volume expansion in normovolemic preterm fetal sheep did not affect blood pressure or cerebral blood flow but was associated with decreased cerebral oxygen delivery. This was further compromised when oxygen content was decreased. We can but speculate on the long-term consequences of these findings in humans, but should be cautious about over aggressive volume correction using crystalloids or colloids which might cause hemodilution and reduce O_2 delivery to the brain.

Because systemic hypotension is associated with increased mortality as well as both short- and long-term morbidity volume expansion, dopamine and dobutamine have been the agents most commonly used to treat hypotension. Norepinephrine is preferred by some clinicians. Dopamine is the most widely used sympathomimetic amine in the treatment of neonatal hypotension, and a Cochrane review confirmed that it is more effective than dobutamine in raising blood pressure [18]. Volume administration is less effective in the immediate postnatal period, and its extensive use is associated with significant untoward effects, especially in preterm infants. During the course of their disease, some of the sickest hypotensive newborns become unresponsive to volume and pressor administration. This phenomenon is caused by the desensitization of the cardiovascular system to catecholamines by the critical illness and relative or absolute adrenal insufficiency.

Corticosteroids have added a new wrinkle to the management of hypotension. Many hypotensive preterm infants have low cortisol levels, and corticosteroids are being used increasingly to prevent or treat hypotension in these babies. Steroids rapidly up-regulate cardiovascular adrenergic receptor expression and serve as hormone substitution in cases of adrenal insufficiency which explains their effectiveness in stabilizing the cardiovascular status and decreasing the requirement for pressor support in the critically ill newborn with volume-and pressor-resistant hypotension. Although corticosteroid therapy has resulted in improved blood pressure and circulation, many complications including unanticipated spontaneous gut perforation, hyperglycemia, hypertension and the long-term problem of increased cerebral palsy and intellectual impairment necessitates judicious use of corticosteroids to support blood pressure in preterm infants [19].

When the etiology of the hypotension is primary cardiac dysfunction (arrhythmia, structural heart disease, myocarditis, asphyxia) the infants may manifest the classical clinical features of congestive heart failure with edema, cardiomegaly, and hepatomegaly. Inotropic agents, with or without peripheral vasodilators, are the treatments of choice. Prostaglandin E1 to restore ductal patency is vital for infants with ductal dependent lesions Structural heart disease or arrhythmia often requires specific pharmacologic or surgical therapy. Fluid boluses may aggravate the condition.

78.8 Hypotension Outcomes

Batton defined low BP as ≤ 25 mmHg in the first 72 hours of life [20]. He followed 67 infants with normal BP, 31 infants with untreated low BP, and 70 infants with treated low BP. Untreated infants with low BP had similar survival rates, but

more cerebral palsy, deafness, or any neuro-developmental impairment when compared with infants with normal BP. Treated infants with low BP had more mortality, and less survival without neuro-development impairment compared with infants who had normal BP. Kiunt also reported that the early need for BP support related to intraventricular hemorrhage, periventricular leukomalacia and major neurodevelopment impairment [21]. On the other hand Pellicer in a prospective evaluation of the effect vasopressors/inotropes for early systemic hypotension on neurodevelopment, found no differences in the rates of abnormal neurologic status, developmental delay, or combined adverse outcome (death or cerebral palsy or severe neurodevelopmental delay between the hypotensive and normotensive groups) [22]

Finally, despite recent advances in our understanding of the pathophysiology and management of neonatal hypotension, there are few data from well conduced trials on the impact of the treatment on organ blood flow and tissue perfusion and on neonatal morbidity and mortality. In view of these significant problems with therapy there is an imperative to better study this problem, define hypotension (based on hemodynamic measurements which are feasible and reproducible) and determine optimal therapy. Such trials are ongoing.

78.9 Hypertension

Hypertension, while not as common as hypotension, can be seen in up to 3% of NICU admissions. As noted earlier, neonatal hypertension is defined as a sustained blood pressure above the 95th percentile using available normative data. BP in newborns increases with both gestational and post-conceptional age as well as with birth weight and the data generated by Zubrow et al [3] provides useful parameters to define hypertension. It is important to ensure that the correct cuff size has been used, with the cuff bladder measuring 2/3 the length of the extremity. Furthermore, the diagnosis should be made based on repetitive measurements (at least three) or consistent elevation of pressure over a 2–3 day period if the infants are asymptomatic.

The estimated incidence of hypertension ranges from 0.2–3% and it is frequently an indicator of other renal or cardiovascular abnormalities [23]. In Cleveland in 1983 hypertension, defined as mean blood pressure greater than 70 mmHg on three separate determinations, was noted in 2% of all neonatal admissions to the intensive and intermediate care nurseries [24]. As most infants are asymptomatic, and the onset is insidious, only by paying close attention to the vital signs, including measurement of blood pressure, will neonatal hypertension be recognized. Measures are then necessary to determine the underlying cause and institute appropriate therapy. In healthy neonates, systolic blood pressure increases rapidly during the first 6 weeks of life with the most rapid rise observed during the first 5 days. A similar pattern is observed for diastolic pressures. The observed increases in blood pressure are positively correlated with birth weight and both gestational and postnatal age [25].

78.10 Clinical Features

In most newborns, hypertension is discovered on routine monitoring of vital signs. These infants are asymptomatic or present with the usual non-specific features in the newborn such as hematuria, feeding difficulties, unexplained tachypnea, apnea, lethargy, irritability, or rarely seizures. Symptomatic infants are often mottled with florid features of congestive heart failure. Skalina in Cleveland reported the rapid onset of a retinopathy identical to that seen in hypertensive adults in neonates with hypertension [26]. Eleven of 21 patients with elevated blood pressure demonstrated some or all of the following abnormalities: increased ratio of venous to arterial caliber, vascular tortuosity (including arteriovenous crossing changes), superficial and deep hemorrhages, and exudates. These findings appeared to resolve after control of the hypertension [26].

78.11 Causes of Hypertension

There are many causes of hypertension (Table 78.2) but in the newborn period, hypertension is primarily is of renovascular origin, although cardiac, endocrine, and pulmonary causes must be considered as well. Neonatal hypertension may be secondary to congenital malformations or due to acquired disease states.

Congenital etiologies include: renal artery stenosis/ hypoplasia, coarctation of the aorta, and abdominal aortic atresia. Renal artery stenosis has also been noted secondary to congenital rubella. Furthermore, there are a number of congenital renal parenchymal abnormalities which have been associated with hypertension in the newborn period. Both autosomal dominant or autosomal recessive polycystic kidney disease (PKD) may present in the newborn period with enlarged kidneys and hypertension. Severely affected infants with PKD are at risk of developing congestive heart failure due to severe malignant hypertension. Hypertension has also been reported in infants with unilateral multicystic dysplastic kidneys. Renal obstruction and hydronephrosis may also lead to hypertension, secondary to impingement on the renal vessels or via the renin-angiotensin system.

Acquired renal parenchymal causes of hypertension in the newborn period include severe acute tubular necrosis and cortical necrosis usually secondary to severe asphyxia. Other acquired causes of neonatal hypertension include: thromboembolic renal artery and renal vein complications secondary to umbilical artery catheterization or to thrombosis associated

Table 78.2 Causes of hypertension

Renovascular conditions
Thromboembolism
Renal artery stenosis
Mid abdominal aortic coarctation
Renal venous thrombosis
Compression of renal artery
Idiopathic arterial calcification
Congenital rubella syndrome
Renal parenchymal disease
Polycystic kidney disease
Multicystic dysplastic kidney disease
Uretero-pelvic junction obstruction
Acute tubular necrosis (birth asphyxia)
Cortical necrosis
Cardio-Pulmonary conditions
Coarctation of thoracic aorta
Bronchopulmonary dysplasia (BPD)
Pneumothorax
Endocrine conditions
Congenital adrenal hyperplasia
Hyperaldosteronism
Hyperthyroidism
Pseudo hypoaldosteronism Type II
Medications/Intoxications
Corticosteroids e.g., dexamethasone
Adrenergic agents
Vitamin D intoxication
Xanthenes – Theophylline/Caffeine
Pancuronium
Phenylephrine
Maternal cocaine or heroin use
Tumors
Wilms' tumor
Mesoblastic nephroma
Neuroblastoma
Pheochromocytoma
Neurologic conditions
Pain
Intracranial hypertension
Seizures
Familial dysautonomia
Subdural hematoma
Miscellaneous conditions
Closure of abdominal wall defect
Adrenal hemorrhage
Hypercalcemia
Extracorporeal membrane oxygenation (ECMO)

with polycythemia or hypercoagulable states. Almost 10% of the infants with indwelling umbilical arterial catheters develop hypertension [27].

Hypertension is also more common in infants with bronchopulmonary dysplasia (BPD), patent ductus arteriosus, or intraventricular hemorrhage as well as those who receive postnatal corticosteroids. Endocrinologic disorders that may produce hypertension in the newborn period include congenital adrenal hyperplasia, hyperaldosteronism, and hyperthyroidism. Salt and water overload and hypercalcemia may also be associated with hypertension in the neonate. Other factors include pain, closure of abdominal wall defects, adrenal hematoma with renal artery compression, seizures, central nervous system disorders including those associated with inherited metabolic disorders, drug-induced hypertension, and infants of drug- dependent mothers with symptoms of withdrawal.

Medications to be aware of include aminophylline, high doses of adrenergic agents, prolonged use of pancuronium, or administration of phenylephrine ophthalmic drops. Hypertension induced by medications typically resolves when the offending agent is discontinued or its dose is reduced. Patients with certain tumors, including neuroblastoma, Wilms' tumor, and mesoblastic nephroma, may also present in the neonatal period. The hypertension results from either compression of the renal vessels or ureters by the tumor or secretion of vasoactive substances such as catecholamines associated with a phaeochromocytoma or neuroblastoma.

There is also a rare disorder, known as hypertension-hyponatremia syndrome, with elevated levels of renin observed in neonates. Renal ischemia from possible microthrombi has been postulated as the precipitating cause and with sodium supplementation and antihypertensive therapy the condition resolves and renin levels return to normal [28].

78.12 Diagnostic Approach

In hypertensive neonates it is important to determine whether any reno-vascular anomalies were detected prior to birth. The history may include drug abuse, perinatal asphyxia, umbilical catheter placement or medications administered to the neonate. It is important to confirm the elevated blood pressure and measure the BP in all four extremities to rule out coarctation of the aorta. The pressures are usually higher in the lower extremity and a differential pressure between the upper and lower extremity would suggest coarctation. During the physical examination a careful search for anomalies, dysmorphic features and careful cardiac evaluation are mandatory. Tachycardia and flushing may indicate a secreting tumor such as a neuroblastoma.

Careful abdominal examination and auscultation over the renal vessels is important as well as a thorough search for features of congenital adrenal hyperplasia, such as sexual ambiguity or hyperpigmentation. Asymmetrical growth (hemi-hypertrophy) together with aniridia would suggest a Wilms' tumor. Remember that BPD is a common non-renal cause of hypertension and these infants tend to manifest the increased blood pressure late in the course of the disease unless they have been treated with corticosteroids [29]. The combination of dexamethasone or aminophylline, together with repeated high doses of adrenergic agents, may all contribute to hypertension.

Table 78.3 Selected drugs for the treatment of neonatal hypertension

Drug	Dose	Comments
Chlorothiazide (diuretic)	IV/PO: 20–40 mg/kg/day divided q 12 hours	Monitor electrolytes
Furosemide (diuretic)	IV: 1 mg/kg/dose q 12–24 hours PO: 1–2 mg/kg/dose q 12–24 hours	Better for short-term use. Monitor electrolytes, Long-term use can lead to osteopenia and renal calculi.
Hydralazine (vasodilator)	IV: 0.1–0.5 mg/kg/dose q 6–8 hours PO: 0.25–1 mg/kg/dose q 6–8 hours	May lead to tachycardia or tachyphylaxis
Amlodipine (Calcium channel blocker)	PO: 0.1–0.3 mg/kg/dose q 12–24 hours	Monitor for arrhythmias, monitor calcium and magnesium
Captopril (ACE inhibitor)	PO: Initial: 0.05 mg/kg/dose, then 0.1–0.4 mg/kg/dose q 6–24 hours	Monitor renal function and blood pressure closely; contraindicated in infants with bilateral renovascular disease
Propranolol (beta blocker)	IV/PO: 0.5–1 mg/kg/dose q 6–12 hours	Monitor blood pressure, heart rate, glucose

The initial evaluation of neonatal hypertension includes determination of serum electrolytes, creatinine, blood urea nitrogen (BUN), and a urinalysis in order to look for renal parenchymal disease. Plasma renin levels are warranted, but are normally high in neonates. Endocrinologic studies including serum and urinary measurements of cortisol, 17 hydroxy-progesterone, aldosterone and thyroxin are carried out when pertinent.

In addition to a chest radiograph, which is helpful to determine cardiomegaly and congestive heart failure, renal ultrasonography is necessary in all hypertensive infants. This will identify arterial and or venous thrombi in addition to renal anomalies. Echocardiography is also useful. Cysto-urethrography is performed when indicated. In selected infants aortography used to be performed but with magnetic resonance imaging and spiral computed tomography (CT) scans this should no longer be necessary. In neonates, nuclear scanning has limited use to determine abnormalities of perfusion because of immature renal function.

78.13 Treatment

Therapy of neonatal hypertension should be tailored to the severity of the blood pressure elevation, and to the underlying cause of hypertension as appropriate. A wide range of therapeutic agents are now available for management of neonatal hypertension in both acute and chronic settings. In most cases hypertension will resolve, but some infants may require prolonged treatment. Pharmacologic management of hypertension in the neonate includes use of beta-blockers, angiotensin-converting enzyme inhibitors, calcium channel blockers, and/or diuretics. Essential elements of therapy also include the avoidance of fluid overload (judicious diuretic therapy may be warranted or fluid restriction if renal function is impaired), relief of pain and discontinuation of medications possibly contributing to the hypertension such as corticosteroids, aminophylline and inotropic agents. Every effort should be made to choose the antihypertensive agent most appropriate for the clinical situation. Blood pressure must be closely monitored as with intravenous therapy the BP may drop precipitously. Examples of available drugs for the treatment of hypertension are listed in Table 78.3. Awareness of their mechanisms of action and side effects are vital in treating these challenging patients. For example, angiotensin-converting enzyme inhibitors such as captopril are excellent antihypertensive agents but are contraindicated in the treatment of babies with coarctation of the aorta, unilateral renal artery disease and hyperkalemia [30]. They must also be used with care in babies with chronic hypertension because an abrupt drop in BP may adversely affect the brain and kidneys,

78.14 Follow-up

The long-term outcomes of neonatal hypertension depend upon the underlying etiology. Although blood pressures can be controlled with antihypertensive medications, infants with parenchymal renal disease may remain hypertensive. When the hypertension is secondary to acquired reno-vascular disease the blood pressure often normalizes over time and medications can be discontinued. However significant structural or functional renal abnormalities may persist. Appropriate follow-up with a pediatrician or pediatric nephrologist must be arranged.

78.15 Conclusions

Although many sick newborns are treated for hypotension and hypertension, the normal physiologic BP range ensuring appropriate organ perfusion is uncertain. Treatment decisions are based on statistically defined gestational and postnatal age-dependent normative BP values, combined with clinical

intuition, because of difficulties evaluating organ perfusion and adequacy of cerebral oxygen delivery. Early onset hypotension is usually due to the combined effects of abnormal peripheral vasoregulation, myocardial dysfunction, and hypovolemia. Volume administration is the primary initial therapy but its use may be associated with significant untoward effects, especially in preterm infants, and should be limited to 10–20 mL/kg of isotonic saline. If the BP cannot be normalized dopamine should be added, sometimes followed by epinephrine and corticosteroids. Hypertension, most often caused by congenital or acquired renovascular disease or volume overload, needs a thorough search for the etiology and cautious treatment so the BP does not fall too quickly or too low.

78.16 Practice Points

- Blood pressure norms are based on statistically defined gestational and postnatal age-dependent normative blood pressure values. Blood pressure increases with increasing gestational age, weight, and postnatal age.
- Treatment decisions for hypotension should be based on a combination of low blood pressure and clinical symptoms. Too much volume is associated with increased morbidity and mortality
- Care must be taken not to drop the blood pressure too quickly in hypertensive neonates as cerebral perfusion pressure will be adversely effected.

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79

Polycythemia and Hyperviscosity

Otwin Linderkamp

79.1 Introduction

The term hyperviscosity cannot be understood without some knowledge of how blood viscosity is defined and measured. Viscosity (V) is a flow property defined as resistance (R) to the movement of blood: R = VZ, where Z is the resistance resulting from the vessel geometry [1]. Blood viscosity is usually measured in flow devices with fixed geometry (e.g., in a rotational viscometer with a defined gap or in a tube viscometer with defined tube diameter and length). In these devices, the blood flow resistance increases linearly with the blood viscosity, and blood flow resistance is measured as pressure (P) or force required to achieve a defined blood flow (F) in the device (V ≈ R = P/F). Blood viscosity decreases with increasing shear forces due to red blood cell (RBC) aggregation at low shear rates and ellipsoidal deformation of RBCs at high shear. In tube viscometers with diameters below 300 μ, blood viscosity decreases linearly with decreasing tube diameter. Without this so called Fåhræus-Lindqvist effect, blood with a hematocrit of 50% and higher could probably not pass through arterioles and capillaries with diameters below 50 μm.

Hyperviscosity is frequently used synonymously with polycythemia (i.e., high hematocrit). However, blood viscosity increases with increasing hematocrit, plasma viscosity and RBC aggregation and with decreasing red blood cell deformability. Neonatal blood has several favorable properties including lower plasma viscosity and RBC aggregation (due to lower concentrations of high molecular weight proteins) and increased RBC deformability [2, 3]. Plasma viscosity is about 20% lower in the neonate compared with adults, thereby decreasing blood viscosity at given hematocrit by 20% [3]. The reduced RBC aggregation decreases blood viscosity at low shear stress and facilitates blood flow at slow flow (e.g., in veins).

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RBC deformability is not a mathematically defined mechanical parameter, but the result of various geometric (shape, volume and excess surface area) and mechanical (membrane elastic moduli, membrane and internal viscosity) properties. Filtration techniques demonstrated decreased filterability of neonatal RBCs due to their large volume [4]. The deformability of neonatal RBCs studied in counter-rotating devices and the RBC membrane elasticity were found increased compared with adult RBCs [5, 6]. However, neonatal RBCs appear to be more sensitive than adult cells to agents that can impair RBC deformability such as acidosis, hypoxemia and bacterial toxins [7–9]. Moreover, neonatal blood contains more RBCs with irregular shape and impaired deformability than adult blood [10].

The viscosity reduction in narrow tubes (Fåhræus-Lindqvist effect) is more pronounced for neonatal RBCs than that of adults (Fig. 79.1) due to their larger volume and increased membrane elasticity. The extent of the viscosity reduction increases with rising hematocrit. Compared to blood viscosity in 500 μm tubes, viscosity reductions of blood with a hematocrit of 0.70 L/L in 50 μm tubes were 50% in term neonates and 39% in adults, whereas the viscosity reductions at a hematocrit of 0.30 L/L were only 29 and 19%, respectively [11]. Because of the enhanced Fåhræus-Lindqvist effect and decreased plasma viscosity, blood viscosity in 50 μm tubes is similar in neonates who have a hematocrit of 0.70 L/L as in adults with a hematocrit of 0.50 L/L (Fig. 79.1). These findings may explain why circulation in neonates is less affected by a high hematocrit than circulation in adults.

79.2 Definition of Polycythemia and Hyperviscosity in Neonates

In the majority of newborn infants, the hematocrit shows marked changes from cord blood levels through the first 24 hours of birth. The changes in the hematocrit depend on the blood volume at birth. In neonates with low blood volume

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

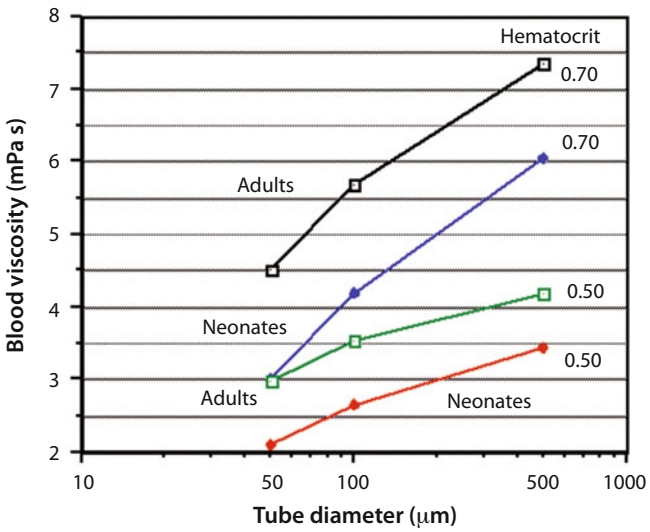

Fig. 79.1 Blood viscosity in adults and full-term neonates measured in tubes with diameters of 50, 100 and 500 μm. The hematocrit was adjusted to 0.50 and 0.70 L/L. Note that in 50-μm tubes the viscosity of neonatal blood with a hematocrit of 0.70 L/L was similar as the viscosity of adult blood with a hematocrit of 0.50 L/L. Adapted from [11]

(e.g., due to early cord-clamping), the hematocrit changes little, whereas in neonates with high blood volume (e.g., after late cord-clamping), the hematcrit rises considerably after birth, reaches the highest values at two hours of postnatal age, and decreases slowly over the next 24 hours [12–14]. Thus, the frequency of polycythemia depends strongly on the time of blood sampling.

The most commonly used definition for neonatal polycythemia is a venous hematocrit of 0.65 L/L or more [15–18]. Screening programs for the detection of neonatal polycythemia are usually based on hematocrit measurements in cord, skin prick or venous blood. Umbilical blood is the preferred source for screening since it is routinely collected for blood gas studies and does not require vessel puncture in the infant. In unselected neonates, the umbilical hematocrit is on average 0.10 L/L higher than that of venous or arterial blood in the infant at 2 hours of birth [12, 13]. Since approximately 50% of unselected infants with an umbilical hematocrit of 0.55 L/L or more develop venous hematcrit values of 0.65% or greater, this value is usually used as indication for peripheral venous blood sampling at 2 hours of birth. However, the umbilical hematocrit is similar in healthy term infants with early and late cord-clamping and, therefore, fails to detect polycythemia after late cord-clamping [14]. The capillary hematocrit measured in unwarmed skin prick blood is on average 0.10 L/L (warmed skin: 0.05 L/L) higher than the venous hematocrit over a wide range of hematocrit values [16, 17]. This has been explained by impaired peripheral circulation and red blood cell trapping in skin vessels [19]. If the capillary hematocrit is 0.70 L/L or greater, a venous specimen should be analyzed.

The definition of polycythemia as a venous hematocrit of 0.65 L/L or more is based on clinical observations in adults who are at high risk of cardio-circulatory disorders such as stroke and heart attack if their hematocrit exceeds this critical value [15]. However, as outlined in the Introduction, neonatal blood demonstrates several beneficial flow properties such as decreased plasma viscosity and increased RBC deformability when compared with adult blood. Moreover, otherwise healthy neonates with hematocrit values of 0.65–0.69 L/L may show some clinical manifestations of polycythemia, but long-term sequelae are rare [3]. For clinical practice the following definitions of polycythemia may be used: 1) a hematocrit of 0.70 L/L or more independent of clinical signs, and 2) a hematocrit of 0.60–0.69 L/L with clinical signs of polycythemia.

Hyperviscosity is usually defined as a blood viscosity (measured by means of a Wells Brookfield Microviscometer) that is two standard deviations greater than the mean of a normal population [12, 16, 17]. Blood viscosity techniques are not generally available. However, blood viscosity in narrow tubes that have diameters of 50 or 100 μm can be calculated from published formulas [11].

79.3 Causes of Polycythemia and Hyperviscosity in the Neonate

Polycythemia in neonates defined as a hematocrit of 0.65 L/L or greater occurs in about 1–5% of all newborn infants [12, 13, 16–18]. Hyperviscosity defined as blood viscosity of more than two standard deviations above population mean of screened neonates occurs in 5% of infants. The frequency of neonatal polycythemia diagnosed in various hospitals depends on whether a screening program is performed, on the mode of cord-clamping and the prevalence of neonates with a high risk for polycythemia. Table 79.1 summarizes causes of and risk factors for polycythemia and hyperviscosity in the neonate.

In infants with late cord-clamping, the incidence of polycythemia is about 25% at two hours, but less than 10% at 24 hours after birth [14, 19–20], since the hematocrit decreases spontaneously after the early postnatal hours (Table 79.2). Blood transfusion from the placenta to the neonate occurs when the umbilical cord is clamped at some time after 5 seconds of birth. Before birth, the fetal blood volume is approximately 70 mL/kg of blood [19, 20]. Late cord-clamping may increase the neonatal blood volume by 35 mL/kg if the newly born infant is kept at or below the level of the placenta. The rapid blood volume expansion is counteracted by extravasation of plasma so that the hematocrit rises from approximately 50% at birth to 58–70% at 2 hours after birth. This increase in hematocrit is associated with a 50% rise in blood viscosity. The Leboyer birth method, which requires placement of the newly born infant on the mother's abdomen and clamping of the cord when it stops pulsating, results in a smaller blood volume expansion due to the smaller pressure

Table 79.1 Causes of polycythemia and hyperviscosity in the fetus and neonate

Increased hematocrit (≥ 0.65 L/L)
- Red cell transfusion:
  - Placenta-to-fetus transfusion: late cord-clamping,* intrauterine asphyxia*
  - Twin-twin transfusion (monochorionic twins)*
  - Maternal-fetal transfusion
  - Transfusion of a large volume of blood or erythrocytes*
- Increased fetal erythropoiesis:
  - Chronic intrauterine hypoxia,* placental insufficiency,* maternal gestosis* or smoking*
  - Small for gestational age* and large for gestational age* infants
  - Endocrine disorders: Hyperinsulinism (diabetic mother,* Beckwith-Wiedemann syndrome*), neonatal thyrotoxicosis,* adrenal hyperplasia*
  - High altitude*
  - Trisomy 13,* 18,* 21*
  - Congenital erythrocytosis
- Dehydration

Increased plasma viscosity and RBC aggregation
- Septicemia (increase in fibrinogen)
- Diabetic mother
- Transfusion of adult plasma or immunoglobulins

Decreased RBC deformability
- Septicemia
- Diabetic mother (glycosylated membrane proteins)
- Hypoxia, acidosis
- Nutritional deficiencies (vitamin E, iron, protein)
- Transfusion of ATP-depleted RBCs

* Discussed indications of screening for polycythemia.

gradient between the placenta and the infant [21]. A recent meta-analysis on effects of late cord-clamping revealed little evidence of harmful effects of late-clamping, but an increased risk for anemia after early cord-clamping [22]. Moreover, neonatal polycythemia resulting from late-cord clamping did not cause developmental or neurologic sequelae during 20 months follow-up [23]. In preterm infants, late cord-clamping appears to result in smaller hematocrit rises than in full-term infants, whereas infants with intrauterine growth restriction or diabetic mothers are at higher risk of developing severe polycythemia after late cord-clamping due to increased intrauterine erythropoiesis [24].

In some disorders such as maternal diabetes and asphyxia, increased plasma viscosity, decreased RBC deformability and increased RBC aggregation may contribute to increased blood viscosity in addition to polycythemia [3]. In septicemia, impaired RBC deformability, increased RBC aggregation and a large number of rigid neutrophils may contribute to impaired micro- and macro-circulation [9].

Transfusion of blood components may cause symptomatic hyperviscosity if the hematocrit rises above a critical limit or the plasma viscosity and RBC aggregation increase as a result of treatment with high doses of immunoglobulins [25]. Because of the higher plasma viscosity of adult plasma, fresh frozen plasma should not be used for hemodilution in polycythemic neonates [26]. RBCs from adult donors are less deformable than neonatal RBCs. Transfusion of adult RBC should, therefore, not result in a hematocrit of more than 0.55 L/L (see Fig. 79.1). Uncontrolled erythropoietin treatment can cause polycythemia if the infant responds particularly well to erythropoietin. However, erythropoietin treatment improves RBC deformability, due to the increased formation of young well-deformable RBC [27].

79.4 Symptoms and Signs of Polycythemia and Hyperviscosity

Increased blood viscosity can increase the flow resistance in various organs, thereby impeding their blood and oxygen supply. Polycythemia in the neonate decreases cardiac output, and blood flow to the brain, the gastrointestinal tract, the kidneys, the lungs, the limbs and the skin [28]. There is concern that this may increase the risk of pulmonary hypertension, renal failure, necrotizing enterocolitis, cerebral ischemia, intracranial hemorrhage, and developmental retardation. However, systemic RBC transport (calculated as product of cardiac output times hematocrit) and RBC transport to the brain (blood flow velocity times hematocrit) remain stable in the neonate over a hematocrit range of 0.40 to 0.70 L/L [29]. Moreover, cerebral oxygenation does not decrease in the neonate up to a hematocrit of 0.70 L/L [30]. At hematocrit of 0.70 L/L and greater, systemic and cerebral RBC transport decrease markedly. This agrees with studies of blood flow in narrow tubes [11].

Clinical consequences of a high blood viscosity are principally a result of impaired circulation and oxygen supply in affected organs (Table 79.3). Central nervous system symptoms such as hypotonia or irritability are of particular

Table 79.2 Effect of cord-clamping on hemorheology in full-term infants at 2 h after birth [14, 21]

	Early [a]	Leboyer [b]	Late [c]	P < 0.05
Hematocrit [L/L]	0.48 ± 0.06	0.58 ± 0.06	0.63 ± 0.05	a < b < c
Plasma viscosity [mPa s]	1.04 ± 0.09	1.06 ± 0.08	1.09 ± 0.09	a = b = c
RBC aggregation	4.2 ± 1.0	4.2 ± 1.8	4.0 ± 1.4	a = b = c
RBC deformation (at 3 Pa)	0.42 ± 0.04	0.41 ± 0.05	0.42 ± 0.05	a = b = c
Blood viscosity [mPa s]	2.8 ± 0.5	3.7 ± 0.5	4.2 ± 0.4	a < b < c

Table 79.3 Clinical features ascribed to polycythemia and hyperviscosity

Central nervous system
- Early effects: hypotonia and sleepiness, irritability and jitteriness
- Neurodevelopment: long-term neurologic and developmental problems
- The reported long-term sequelae are mostly caused by underlying condition as intrauterine and perinatal asphyxia or maternal diabetes

Heart and lungs
- Tachycardia, tachypnea, respiratory distress
- Cyanosis, plethora
- Chest radiography: cardiomegaly, pulmonary plethora
- Echocardiography: increased pulmonary resistance, decreased cardiac output

Gastrointestinal tract
- Poor suck, vomiting
- Necrotizing enterocolitis

Kidneys
- Oliguria (depending on blood volume)

Metabolism
- Hypoglycemia
- Hypocalcemia
- Jaundice

Hematology
- Mild thrombocytopenia
- Thrombosis

concern, since they may indicate increased risk for long-term neurological and developmental sequelae. Peripheral or systemic cyanosis and plethora are merely signs of a high hematocrit, but not of compromised circulation. A decrease in platelet count has been suggested as an indicator of polycythemia [31], but is also observed in infants with growth retardation and in infants of mothers with gestosis or smoking during pregnancy.

Reported frequencies of clinical manifestations in polycythemic neonates vary widely. This may be explained by inclusion of neonates who have a high risk of perinatal complications independent of the occurrence of polycythemia (e.g, asphyxia, intrauterine growth restriction, diabetic mother, malformations), and by inclusion of different numbers of neonates who have venous hematocrits of 0.70 L/L or greater. Wiswell et al. [32] observed clinical signs and symptoms in 50% of neonates who had hematocrits of 0.65 L/L or greater. Van der Elst et al. [33] found most polycythemic infants healthy and unaffected. Moreover, most clinical signs and symptoms of polycythemia are of minor importance and can be observed in neonates who do not have polycythemia. Moreover, some complications such as thromboses or necrotizing enterocolitis may be caused by the exchange transfusion procedure rather than by the polycythemia.

Long-term studies of the incidence of developmental and neurological abnormalities at 1–7 years also reach conflicting conclusions [3, 34]. Goldberg et al [35] reported a high incidence of neurological problems at 9 months among infants who had polycythemia. Black et al [36, 37] observed that neonatal polycythemia was associated with a high risk of neurologic and developmental problems at 1 and 2 years. At 7 years only small (insignificant) differences were observed between children with and without neonatal polycythemia. However, both groups included infants who had additional risks for developmental problems. Moreover, hemodilution did not influence the results significantly. A study performed in Thailand demonstrated that impaired outcome at 1–2 years of age was caused by intrauterine hypoxia rather than by neonatal polycythemia [38]. Investigators who included only polycythemic infants without additional risks found no effect of polycythemia and hemodilution on long-term outcome [23, 39–40]. Drew et al [41] reported that increased blood viscosity was a better predictor of poor outcome than was a high hematocrit.

79.5 Prevention and Treatment of Polycythemia and Hyperviscosity

Prevention of polycythemia includes prevention of risk factors such as poor control of maternal diabetes and intrauterine asphyxia. If the risk of polycythemia is increased, the umbilical cord should be clamped immediately after birth to avoid placental transfusion [24]. However, late cord-clamping in otherwise healthy neonates should not be avoided for concern of polycythemia, since overall late cord-clamping is more beneficial for the normal neonate than early cord-clamping [20, 22].

Since the hematocrit decreases spontaneously subsequent to the peak value at 2 hours of birth, it appears reasonable to accelerate this natural hemodilution by infusion of normal saline. Although several text books recommend “liberal fluid intake” for polycythemic infants, no study using the efficacy of this approach has been found in the literature. According to our own experience, the infusion of 10 mL/kg of normal saline over 1 hour decreases the hematocrit below the critical level in approximately 50% of infants.

The general recommendation for the treatment of polycythemia is partial exchange transfusion (isovolemic hemodilution) via an umbilical vein catheter or using a radial artery for blood withdrawal and a scalp or peripheral vein for blood replacement [42]. Isovolemic hemodilution in neonates is performed via an umbilical venous catheter using 5% human serum albumin, serum (free of activated clotting factors), or crystalloids (normal saline or Ringer solution). Adult plasma increases the plasma viscosity and the RBC aggregation and, therefore, should not be used for hemodilution [26]. De Waal et al [43] conducted a systematic review to determine the efficacy of crystalloid (normal saline or Ringer solution) versus colloid (plasma or 5% albumin) for hemodilution. No meaningful differences in effectiveness (decrease in hematocrit, relief of symptoms) between plasma, 5% albumin and

crystalloid solutions were observed in six studies. Plasma expanders such as hydroxyethyl starch and Hemaccel have been used for hemodilution in neonates [44], but little is known about their distribution and metabolism in the neonate.

The use of umbilical vein catheterization for partial exchange transfusion may cause severe complications including septicemia, vasospasm, vessel perforation with hemoperitoneum or intrahepatic hematoma, air embolism, arrhythmia, thrombosis, portal hypertension and necrotizing enterocolitis [42]. The exchange transfusion itself may cause blood volume and pressure fluctuations, electrolyte abnormalities, hypoglycemia, thrombocytopenia and hemolysis. The stress resulting from the procedure may explain that the heart rate [17, 29] and energy expenditure [45] may rise after hemodilution for neonatal polycythemia. Exchange transfusion with plasma via the umbilical vein increases the risk of necrotizing enterocolitis [16].

Since the central hematocrit is 0.65 L/L or greater in about 3% of all neonates, several thousand otherwise healthy neonates may be exposed to this potentially harmful invasive procedure of partial exchange transfusion if this threshold is used as indication, although long-term studies of children who had neonatal polycythemia failed to show the benefits of exchange transfusion for hemodilution [3, 34, 46]. Hemodilution, therefore, is presently recommended if the hematocrit is 0.70 L/L or higher or if symptoms of compromised circulation are present at hematocrits of 0.60 to 0.69 L/L.

The following recommendations for the treatment of neonatal polycythemia are widely used.

- Screening for polycythemia may be done in small and large for gestational age infants, infants of diabetic mothers and some of the rarer risk factors listed in Table 79.1. It can be performed in umbilical blood, in skin prick ("capillary") blood, venous blood taken without prolonged tourniquet or excessive squeezing, and in arterial blood.
- If symptoms of polycythemia are observed, other explanations should be explored before an exchange transfusion is performed. The laboratory work-up includes measurements of hematocrit, blood gases, leukocyte and platelet count, CRP, serum glucose and calcium (Table 79.2). Pulse oximetry and echocardiography may be indicated. Hypoxia, acidosis, hypoglycemia and electrolyte abnormalities should be corrected.
- If the venous or arterial hematocrit is 0.65–0.69 L/L and signs of impaired circulation are present or if the hematocrit is 0.70 L/L or greater with or without signs, an infusion with 10 mL/kg of normal saline is given in 1 hour, and the venous hematocrit is determined again.
- If the hematocrit remains above the critical level, exchange transfusion is performed with normal saline or Ringer solution. Cristalloids are as efficacious as colloids (plasma, 5% albumin and plasma expanders) for hemodilution.
- In infants with high risk of impaired RBC deformability (e.g., diabetic mother), hemodilution may be indicated in some symptomatic infants with a hematocrit of 0.60–0.64 L/L.
- Informed consent of the parents must be obtained as efficacy and safety of exchange transfusions for polycythemia are uncertain.
- The exchange transfusion can be performed via an umbilical vein catheter or a radial artery for blood withdrawal and a routine venous infusion for blood replacement.
- The volume of serum exchanged is:

  $$\text{Volume} = 100\ \text{mL/kg} \times (\text{observed} - \text{desired hematocrit})$$

  where 100 mL/kg is the total blood volume in polycythemic infants and the desired hematocrit is usually 0.55 L/L. Usually an exchange volume of 20–30 mL is satisfactory. In each single withdrawal 1–1.5 mL/kg of blood is removed over 2–3 minutes and replaced over another 2–3 minutes.
- Infants should be monitored during the procedure and for several hours thereafter.

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80

Patent Ductus Arteriosus

Bart Van Overmeire

80.1 Epidemiology

Patent ductus arteriosus (PDA) is the most common cardiac abnormality of the preterm infant. Its incidence is inversely related to gestational age, such that it affects almost 60% of infants less than 28 weeks' gestation. Data published in 2007 demonstrated that spontaneous closure of the ductus occurs in 30% of infants with birthweights below 1500 g (very low birth weight [VLBW]) [1]. It is obvious that percentages are decreasing for each lower birthweight category (Table 80.1), but also that a markedly wide variation exists among centers. This may reflect the influence of varying factors on the closure of the ductus.

Higher closing rates, and as a consequence lower incidences of PDA, have been reported in several papers for European infants. A randomized comparative multicenter trial that compared early (day 3) versus late (day 7) indomethacin treatment for PDA in preterm infants with gestational ages between 26 and 31 weeks and suffering from respiratory distress syndrome (RDS), revealed that 281 of 380 (74%) spontaneously closed their duct by the age of 7 days [2]. In 210 infants who received placebo in a double blind randomized trial of ibuprofen prophylaxis, high spontaneous closing rates on the 3rd to 4th day of life were confirmed by echocardiographic assessment [3]. Data are displayed for five birthweight and gestational age categories (Table 80.2).

Data from the neonatal registry of the Belgian college of physicians, demonstrate a closed or non-symptomatic PDA in 46.7% of 2635 VLBW infants for the years 2007-2009 [4]. As compared to these numbers, the closure rates described by Koch et al [5] in the United States seem lower, e.g., 34% on the third day of life in a group of 122 infants with birth weights below 1000 g. Other US data report 31% closure below 1000 g and 67% in a group of 65 VLBW infants [6]. It is clear that a wide variation exists in spontaneous ductal closure in preterm infants. In addition to immaturity, which is the factor that contributes most to PDA, various cofactors affect the occurrence of PDA. For many years, the presence of severe RDS has been recognized as a delaying factor for closure [7], as is the association with sepsis [8]. Intrauterine growth restriction also affects the behavior of the ductus [9]. The group of Rakza demonstrated convincingly that hypotrophic infants had significantly larger left to right shunts appearing earlier after birth and needing earlier treatment than eutrophic controls [10]. The authors explain that chronic hypoxia in utero may have caused a faster decrease of the pulmonary vascular resistance through the production of higher noradrenaline levels. Additionally, major anomalies of both the intima and media of the ductal vessal wall have been described [11]. It is a common belief that excessive fluid administration during the first days of life is associated with the occurrence of PDA. Unfortunately, poor evidence exists to support this view. A systematic meta-

Table 80.1 Closed or non-symptomatic PDA among VLBW infants surviving >12 hours

501–750 g	750–1000 g	1001–1250 g	1251–1500 g
n = 4,046	n = 4,266	n = 4,557	n = 5,284
51 (17–80)	62 (40–89)	77 (52–91)	87 (67–93)

Data expressed as % (center range) from [1].

Table 80.2 Spontaneous closure of PDA in 210 VLBW infants

Birthweight (g)	Closed ductus	Gestational age (w)	Closed ductus
500–750	44%	24–25	54%
751–1000	62%	26–27	54%
1001–1250	60%	28–29	60%
1251–1500	74%	30–31	79%

Data from [3].

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

analysis on this topic was last updated in 2008. Four of the five included trials date from the era before antenatal steroids and exogenous surfactant therapy and the one from 1999 shows very weak evidence. Nevertheless, the authors conclude that the practice of restricting water intake to physiological needs in preterm infants might be expected to decrease the risk of PDA [12]. The role of cortisol was highlighted by the studies of Watterberg demonstrating an inverse relationship between serum cortisol levels on postnatal days 3–4 and 5–6 and the occurrence of PDA [13]. It is known that both antenatal [14, 15] and postnatal steroid administration are associated with lower incidences of PDA [16, 17]. The prenatal use of NSAIDs as tocolytics has an impact on the postnatal reactivity of the ductus. Indomethacin and ibuprofen freely cross the placenta [18, 19]. During pregnancy they have a vasoconstrictive effect on the ductus and fetal pulmonary circulation which increases with advancing gestational age. Antenatally induced changes in the fetal ductal wall lead to a decreased reactivity of the ductus to postnatal NSAID treatment with an increased rate of treatment failure [20, 21]. The use of magnesium sulfate in pregnant women has been associated with a higher risk of developing PDA in extremely low birth weight infants [22] and with a reduced responsiveness to indomethacin prophylaxis [23]. Other large studies did not confirm these observations [24].

Many pharmacologic agents that are commonly used in the early neonatal period may influence the behavior of the ductus. Although caffeine has no direct effect on preterm sheep ductus' contractility [25], markedly less treatment for PDA was observed in the group of infants who received caffeine in a large multicenter clinical trial including 2,006 subjects [26]. In contrast to what was suggested years ago, there actually seems less evidence for a reducing effect of the postnatal administration of thyroid hormone [27] and the shielding of the thorax during phototherapy [28] on the occurrence of PDA. The administration of furosemide, by increasing circulating prostaglandin E2 levels, has been associated with an increased incidence of PDA in a study of 66 infants with RDS [29]. Six of seven studies that investigated the use of furosemide in preterm infants with RDS of less than 5 days of age were performed before the systematic application of prenatal steroids, surfactant or fluid restriction. However, a systematic review combining the trials indicated an increased risk of PDA and hypovolemia [30]. More recent data on the concomittant use of furosemide during NSAID treatment for PDA underscore the risk of inducing transient renal failure [31] but do not confirm the clinical effect of delaying the closure of the ductus [32, 33].

Various other frequently used drugs in the NICU may have unexpected vasodilatory actions on the ductus. To date, no clinically relevant effect on the behavior of the ductus has been described by the inhalation of nitric oxide [34–36]. Aminoglycosides, cimetidine, ranitidine and heparin are other possible candidates with dilating effects on the ductus meriting further prospective evaluation [37].

80.2 Physiology and Pathogenesis

During fetal life the patency of the ductus is essential. It allows 90% of the right ventricular output to bypass the high-resistance pulmonary vascular bed. Fetal patency of the ductus before birth and the closure after birth is the result of a balanced interaction of locally produced and circulating mediators, low oxygen tension and the unique structure of the vessel wall. Prostaglandins, converted from arachidonic acid by COX enzymes, play a major role in maintaining ductal patency in utero. There are two separate genes encoding COX proteins, COX-1 and COX-2, and a variant of COX-1 informally termed COX-3 has also been identified [38]. COX-1 is mostly constitutive whereas COX-2 is highly expressed during inflammation. COX-2 has been found to be increasingly expressed in the fetal ductus arteriosus with advancing gestation and is the main contributor to local PGE_2 generation in the ductus at term [39]. Of the five major prostanoids (PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 and TXA2), predominantly PGE_2 and PGI_2 have high levels in the fetus because of high placental production and low clearance by the fetal lungs. They are the most potent ductal relaxants.

The increase in postnatal oxygen tension along with a decreased sensitivity of the ductus to PGE_2 as the fetus approaches term, initiate the closure of the ductus [40]. In the term infant constriction begins within the first few hours after birth and functional closure is usually completed within 24–48 hours of age.

After the initial functional constriction, a phase of neointimal thickening and remodeling occurs, leading to anatomical occlusion after several days [41]. Recent experiments have shown that platelets play a crucial role by promoting a thrombotic sealing and supporting luminal remodeling for the definite closure of the ductus [42, 43].

In the preterm infant the closure usually takes longer. The immature ductus has an increased sensitivity to PGE_2 and its constriction after birth is weaker. At comparable O_2 concentrations the ductus of the very immature fetus generates far lower tensions than those observed in the near-term fetus precluding definitive closure. NO synthase expression in the vasa vasorum of the vessel media is further increased and impedes the critical degree of contraction which is essential to elicit tissue hypoxia, initate remodeling and ultimately definitive closure of the ductus [44]. This carries an increased risk of reopening. A similar mechanism is believed to contribute to the failure of ductal closure of fetus previously exposed to maternal indomethacin [45]. In addition, there is evidence that the major vasorelaxant nitric oxide (NO) plays a role in the very immature ductus. Inhibition of NO synthase was found to exert a greater effect in closing the ductus of the more immature fetus whereas COX inhibition exerted a greater effect in the near-term fetus. The combined treatment with NO synthase and COX inhibitors was more effective than simply using COX inhibitors, but severe side effects precluded the introduction of this approach in clinical practice [46].

80.3 Pathophysiology

Almost all morbidity associated with PDA in preterm infants may be explained by the persistent left to right shunt through the vessel, causing a systemic hypoperfusion and a pulmonary hyperperfusion. In most infants, the heart can cope with the increasing demands by increasing left ventricular stroke volume and heart rate.

80.3.1 Pulmonary Effects

The lungs receive an increased perfusion which may lead to decreased lung compliance, lung edema, and increased risk of pulmonary hemorrhage [47, 48]. Symptoms and signs appear as the limits of compensatory mechanisms are reached. An association between the duration of the left to right shunt and the development of bronchopulmonary dysplasia has been demonstrated [49], although the simultaneous presence of an infection largely increases the risk [8]. Unfortunately, in studies of prophylactic treatment of PDA the incidence of BPD was not reduced. This may be explained by the study design, as all infants in the control group were given a back-up treatment only 1–3 days after the randomization. Alternatively, indomethacin may promote BPD in itself by inducing moderate renal dysfunction leading to increased lung fluid [50]. Experiments in baboons confirmed that a negative influence of a moderate left to right shunt increases clearly pulmonary to systemic flow ratio and exerts a negative effect on pulmonary mechanics. The most striking observation was an arrested alveolar growth and a reduced alveolar branching causing a significantly lower alveolar surface area and complexicity in the subjects that were exposed to persisted shunting [48].

80.3.2 Systemic Effects

Other organ systems may suffer hypoperfusion, including the gut, kidneys and the brain. A persistent patency of the ductus has been shown to be a risk factor for increased mortality. [51, 52]. Disturbances in renal perfusion have been shown [53]. The decrease in mesenteric blood flow [54, 55] has been associated with increased risk of gut ischemia, necrotizing enterocolitis [56], and causing a prolonged interval to full feeds [57]. In infants with PDA the superior mesenteric artery flow velocities were significantly lower postprandially as compared to those with closed duct [55].

80.3.3 Cerebral Effects

Already in the early 1980s hypoperfusion of the neonatal brain as a result of ductal left to right shunt was demonstrated by Doppler flow studies [58] and confirmed repeatedly on many occasions with other imaging techniques [59, 60].

More recently, an impressive lowering of the regional oxygen saturation in the brain could be visualized in infants with a moderate ductal shunt by use of near infrared spectroscopy (NIRS), and a prompt amelioration after closure of the ductus [61]. Although PDA has been clearly associated with all above-mentioned morbidities, in many situations, its causal role is not straightforward.

80.4 Pharmacological Treatment

Because left to right shunt through the ductus is associated with increased morbidity and mortality and because prostaglandins play a major role in patency of the ductus, for decades cyclooxygenase inhibitors have been used to treat PDA [62]. Successful closure of the duct has been reported to occur in 40–80% of treated infants. The risk of reopening increases to above 20% in the smallest infants. Because the process of intimal cushion formation during closure of the duct is inhibited by blockade of prostaglandins in the most immature infants, COX inhibitors are paradoxically less effective in extremely preterm neonates [63, 64]. Until 2004, indomethacin was the only COX inhibitor approved for treatment of PDA in most countries. Since late 1990s ibuprofen has been studied as an alternative for the treatment and prophylaxis of PDA.

The decision to start with pharmacological treatment in an individual infant depends on many factors. It is obvious that if factors in favor of a spontaneous closure of the duct are present, many clinicians will postpone pharmacological treatment by a few days. The appearance of signs and symptoms in the lungs or other organ systems should motivate to the initiation of more prompt treatment.

Schematically, three approaches are possible. Firstly, a conservative management with restriction of fluids combined with optimized respiratory support including positive end expiratory pressure; secondly, the pharmacological treatment with either ibuprofen or indomethacin; and finally surgical ligation of the ductus. Benefits and risks of each approach should be balanced, taken into account the specific characteristics of the infant such as birth weight, postnatal age, antenatal exposure to corticosteroids or NSAIDs, and the presence of comorbidities or infection.

80.4.1 Hemodynamic Significance of the PDA-shunt

Because invasive catheterization is not possible in small preterm infants, echocardiographic assessment is the method of choice to evaluate ductal patency and magnitude of PDA

shunting [65]. The technique allows monitoring the effects of intervention and has been used to guide both doses and the duration of pharmacological treatment [66–68]. It is generally accepted that a left atrium-to-aortic-root diameter ratio of ≥ 1.4 in the parasternal long axis view, a ductal diameter of 1.4 mm/kg body weight, and flow reversal in diastole in the descending aorta, indicate a hemodynamically significant left to right shunt [69]. When the mean end diastolic flow velocity in the left pulmonary artery exceeds 0,2 m/sec, there needs to be an additional high index of suspicion of significant left to right shunt [70].

Because frequent echocardiographic studies may destabilize the infant and in some NICUs it is not easy to obtain a bedside cardiac ultrasound, biomarkers such as B-type natriuretic peptide (BNP), NT-pro-BNP and cardiac troponin T (cTNT) are now more frequently used to detect "symptomatic" PDA and to guide treatment [71–74]. BNP is a vasoregulatory peptide that is released by the ventricles in response to increases in cardiac volume and pressure [75]. BNP levels did correlate very well with the magnitude of the left to right shunt as examined by echocardiography and reflected the moment of closure. Nevertheless, the high variability in the BNP measurements preclude their use as monitoring for changes in shunt magnitude [76]. The combination of measurement of biomarkers as a screening tool in those centers where echocardiography is not readily available, a high clinical suspicion and a comprehensive cardiac ultrasound and Doppler assessment, is probably an optimal approach to guide timely treatment of PDA [77].

80.4.2 Prophylaxis

A vast amount of studies have investigated the timing of pharmacological treatment of PDA. Prophylactic, early pre-symptomatic and symptomatic treatment strategies have been compared [78]. More than 30 studies and reviews addressed prophylaxis with non-steroidal anti-inflammatory drugs (NSAIDs) in preterm infants. The last updated meta-analyses available demonstrate that both prophylactic indomethacin and ibuprofen reduce significantly the risk of developing a symptomatic PDA and the need for ductal ligation [79, 80]. Nineteen prophylactic indomethacin trials were eligible in which 2,872 infants were included. The incidence of symptomatic PDA was very significantly reduced: typical relative risk (RR) 0.44; 95% confidence interval (CI) 0.38–0.50. In the studies eligible for the ibuprofen review the ductus had closed spontaneously in 58% of the infants of the control group by the third day of life. Based on available data the prophylactic administration of ibuprofen cannot be recommended. Indomethacin offers the additional effect of reducing the occurrence of intraventricular hemorrhage (IVH) (typical RR 0.66 95% CI 0.53–0.82). No positive effect on any other outcome parameter could be demonstrated. In particular, no decrease in the rates of bronchopulmonary dysplasia, death, necrotizing enterocolitis, or white matter disease. Notwithstanding the decrease of IVH, no improved neurodevelopmental outcome has been demonstrated [80, 81].

80.4.3 Treatment

Indomethacin and ibuprofen have also been extensively studied for the treatment of PDA. Ibuprofen disturbs significantly less regional circulations, which may offer less dysfunction of kidney, intestines, and brain [82–84]. Urine production was less affected in preterm infants (n = 148) that were treated on day 2–3 of life with ibuprofen than in those receiving indomethacin [85]. This observation resulted in ibuprofen being introduced for clinical use in preterm infants. Necrotizing enterocolitis was diagnosed twice as often in the indomethacin group (8 versus 4; p = 0,37) [85]. Cerebral oxygen availability has repeatedly been shown to be less affected with ibuprofen [83, 86]. Systematic reviews of the trials that evaluated indomethacin or ibuprofen for early treatment of a non-symptomatic or symptomatic PDA confirmed a comparable efficacy of both drugs [87]. The most recently updated systematic meta-analysis included 20 studies [88]. There was no statistically significant difference between indomethacin and ibuprofen efficacy or in failure to close the ductus in 19 comparative studies including 956 infants with a typical relative risk (RR) of 0.94 (95% CI 0.76–1.17). Infants receiving ibuprofen treatment have less evidence of transient renal failure and less risk of developing necrotizing enterocolitis: 15 studies including 865 infants; typical RR 0.68 (95% CI 0.47–0.99). No other differences were noted, e.g., mortality, reopening of the ductus, need for ligation, duration of ventilatory support, duration of supplementary oxygen and development of BPD.

The conventional doses of indomethacin and ibuprofen are different, based on their respective pharmacokinetic parameters. Adapted dosing schemes have been tried to augment efficacy/side effect ratio of indomethacin, however, with variable success [89] (see also § 80.4.5).

80.4.4 Risks of Treatment

Indomethacin and ibuprofen are not devoid of adverse drug reactions. Due to their vasoconstrictive effects, both drugs affect the perfusion of various organ systems to a certain extent, e.g., the gut, kidney and brains. Although ibuprofen initially seemed to have a more favorable safety profile with less disturbance of regional circulations [82–86], the occurrence of unexpected hypoxemia caused a lot of concern. Acute pulmonary hypertension developed in three preterm infants immediately after the infusion of ibuprofen THAM-buffered solution [90]. A clear explanation for this phenomenon is lacking. One infant of 169 treated with ibuprofen-lysine presented

the same adverse effect, but no causal relationship could be found between PPHN and ibuprofen-lysine in another 229 treated infants [3, 91, 92]. Additional studies could not confirm a clear association between the use of ibuprofen-lysine and severe hypoxemia. Although the trial that first reported this serious adverse effect was halted by the national authorities [93], the ibuprofen formulation under investigation became registered for clinical use in Europe in 2004. Ibuprofen-lysine was registered for treatment and prophylaxis of PDA in preterm infants in the US in 2006. Ibuprofen is insoluble in water and more than 90% bound to serum albumine [94]. An *in vitro* model demonstrated that at higher ibuprofen concentrations (750 micromol/L or 150 mg/L) in infant serum containing bilirubin, the fraction of unbound bilirubin increases fourfold [96]. This displacement of bilirubin from albumin binding sites may be clinically relevant in jaundiced preterm infants, as high levels of free bilirubin are associated with brain damage, hearing loss and kernicterus [96, 97]. When ibuprofen is administered in the recommended dosages of 10, 5 and 5 mg/kg at 24 hourly intervals to neonatal infants, ibuprofen serum peak levels of 20–40 mg/L are reached [98]. According to the *in vitro* model, an increase of 10% of the free fraction of bilirubin can be expected at such ibuprofen levels. Three smaller studies exploring the rise of unbound bilirubin were rather reassuring [99–101].

Additional risks of NSAID use for closing PDA in preterm infants are the occurrence of an isolated ileal perforation, particularly when there is concomitant use of steroids [102, 103]. Although predominantly reported for indomethacin, the risk may be similar for ibuprofen as its mechanism is related to microvascular changes and not to disturbances of the regional perfusion of the gut [104]. Increased bleeding tendency has been described after indomethacin use [105], but except for some increased occult blood appearing in stools this does not seem to cause clinically relevant problems. Some neonatal intensive care units are reluctant to continue or introduce enteral feedings during NSAID treatment. Eighty percent of the infants that participated in the European trials of prophylactic and early therapeutic NSAID for PDA were receiving trophic feedings without any apparent untoward effect [2, 3, 85, 93]. The most recent meta-analysis showed that necrotizing enterocolitis is less likely to occur post ibuprofen than post indomethacin treatment [88].

80.4.5 NSAID Dosing Schemes

The pharmacokinetic parameters vary widely in preterm infants due to physiological changes that occur after birth. Studies have shown large prolonged half-life for indomethacin (11–36 h) and reduced clearance rate in the preterm infant, both of which reveal marked interindividual variations [106, 107]. The volume of distribution also varies and is influenced by the patency of the ductus [108]. Accordingly, optimal therapeutic dosing is difficult to establish [109]. The most widely used doses for indomethacin are three times 0.1 to 0.25 mg/kg administered every 12–24 hours. The lower dose with longer interval is recommended when treatment is initiated on the first day. The registered dosing scheme for ibuprofen consists of a first dose of 10 mg/kg followed by a second and third dose of 5 mg/kg at 24 hourly intervals.

In order to improve efficacy/side effect ratio of NSAID during treatment for PDA, a variety of adapted dosing schemes have been studied. As it has been observed that PGE2 production resurges within 5 days of indomethacin treatment [110], a prolonged course consisting of additional indomethacin doses has been tried [89]. Unfortunately, no significant benefit for closure rates, not less reopenings or ligations, and no improvement for BPD, IVH or mortality rates has been obtained. A lower proportion of infants with diminished urine output was observed (typical RR 0.27; 95%CI 0.13 –0.6) but at the expense of an increased risk of NEC (RR 1.87; 95% CI 1.07–3.27) [89].

The continuous infusion of indomethacin has been advocated to avoid disturbances in cerebral perfusion [111], but was reported later to be less effective in extremely low birthweight infants [112]. Interestingly, by applying a stepwise increasing dosage of indomethacin based on echographic evaluation, the group of Sperandio et al [66] obtained ductal closing rates up to 80% without apparently increasing side effects. After an initial standard treatment, subsequent doses were given if the ductus persisted as assessed by echocardio-Doppler. Cumulative indomethacin doses were as high as 6 mg/kg [66]. As the efficacy of ibuprofen at extremely low gestational ages is comparably reduced to indomethacin [113], the administration of additional doses has been investigated. In a group of 25 VLBW infants that received a second course of three doses of ibuprofen the additional closure was 48 % [114]. Su et al [113] obtained an additional closure rate of 50% by administrating up to six doses of ibuprofen. Similar efficacy was demonstrated after the first and second course in a study population of 160 infants with birthweight below 1000 g with a cumulative closure rate of 65% [115]. In order to optimize the efficacy of ibuprofen, an adapted dosing scheme based on postnatal age has been proposed [116]. Further studies are warranted to investigate whether an individualized ibuprofen dosing scheme would result in an increased benefit/risk ratio.

80.5 Surgical Treatment

Since the introduction of indomethacin in the 1980s, surgical ligation has been reserved for those infants for whom treatment with NSAIDs fails or is contraindicated [117]. Beyond the third to fourth week of life, efficacy of COX-inhibitors rapidly declines as the patency of the ductus is less regulated by prostaglandins. Ligation then becomes an option, however

it remains nonetheless associated with a number of complications such as pneumothorax, bleeding, IVH, chylothorax as a result of thoracic duct injury, vocal cord paralysis, wound infection, hypotension, and left ventricular dysfunction in the days after the procedure [118–122]. The morbidity rate ranges form 1 to 16% and mortality from 0 to 10%. In a large retrospective study, surgical ligation and indomethacin treatment for significant PDA were associated with a comparable risk for NEC or NEC-related gastrointestinal complications [123] but mechanics of breathing significantly improved after ligation [124]. However, there are no recent controlled comparisons between pharmacological, conservative and surgical methods of PDA closure. Only one trial in 1983 enrolling 154 infants, compared initial surgical ligation versus medical treatment [117]. No statistically significant difference was found between the groups for mortality, chronic lung disease, NEC or IVH. An increase in pneumothorax (RR = 2.68; CI 1.45–4.03) and retinopathy of prematurity (RR = 3.80; CI 1.12–12.93) was observed in the ligated group. The use of prophylactic ligation cannot be recommended based on the limited current evidence [125]. Additional data from the TIPP study indicated that ligation of the ductus was associated with a higher risk of BPD, severe retinopathy of prematurity and neurosensory impairment [126]. A retrospective analysis of 446 infants showed that ligation was significantly associated with chronic lung disease [127]. In a group of 20 infants, a further decrease of oxygenation of the brain was observed by use of near infra red spectroscopy [128]. No increased brain injury, however, was noted in a preterm baboon model [129]. Because of the associated risks of ductal ligation in preterm infants in recent papers and the absence of recent comparative clinical trials, the primary surgical closure of the ductus does not seem advisable (130).

80.6 Conclusions

PDA continues to be a frequent complication of extremely premature birth, despite the more generalized use of antenatal steroids, postnatal surfactant administration and the improvement of non-invasive ventilatory strategies. Many perinatal factors influence the development of a symptomatic PDA. A large left to right shunt may cause profound hemodynamic disturbances to local organs and the brain. From the NSAIDs studied, ibuprofen actually seems to be the preferred drug for pharmacological treatment because of its more favorable benefit/risk ratio. The therapeutic response of the most immature infants, however, is limited for all investigated COX-inhibitors. Surgical ligation of the duct should be considered as a back-up treatment, because it carries the risk of serious complications and destabilization of the infant. Unfortunately, in spite of many studies, investigations and trials, there is no generally accepted and conclusive evidence to decide at which moment and to which infants the treatment of the ductus should be pursued in order to improve their long-term outcome.

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81

Bilirubin Metabolism, Unconjugated Hyperbilirubinemia, Physiological Neonatal Jaundice

Giovanna Bertini and Carlo Dani

Jaundice is the most frequent condition identified in a newborn. Recent data show that about 25% of neonates have visible jaundice during their hospitalization, and 5.1% have a serum total bilirubin (STB) concentration >12.9 mg/dL (220.59 μmol/L) [1].

81.1 Bilirubin Metabolism

Hyperbilirubinemia is defined as a STB >20 mg/dL. It is caused by a high rate of production of bilirubin or reduced hepatic clearance or increased intestinal absorption. Bilirubin is mainly produced from the catabolism of hemoglobin, and partially from non-erythroid heme-proteins, myoglobin, and ineffective erythopoiesis (Fig. 81.1). The site of conversion of heme to bilirubin is the reticulo-endothelial system, and together with bilirubin, a molecule of carbon monoxide (CO) and an atom of iron are the products of the catabolism (Fig. 81.1). The determination of CO in the expired air or the measurement of CO-hemoglobin are able to quantify the production of bilirubin in a newborn [2]. Bilirubin production in a normal neonate is 2 or more times greater that in the adult per kg bodyweight. In addition, a transient deficiency of bilirubin conjugation is present at birth (during fetal life bilirubin is cleared by the mother through the placenta), and disorders of hepatic uptake and intrahepatic circulation have been demonstrated [3].

Bilirubin is insoluble in water, but in the blood is transported bound to albumin at 2 binding sites (one at very high affinity) in equilibrium with a very low concentration of unbound or free bilrubin [4]. About 8 mg of bilirubin is bound to each gram of albumin. Bilirubin is conjugated to monoconjugate and diconjugated bilirubin by the enzyme system UDP-Glucuronosyltransferase (UGT) 1A1 codified by the UGT 1A1 gene complex. When bilirubin load in newborns is high, bilirubin is mainly conjugated with only one molecule of glucuronic acid. In the intestines, monoconjugated bilirubin is more easily deconjugated by β-glucuronidase and reverted to native bilirubin which can be absorbed by the gut. In fact, the intestine of neonates is almost sterile, and bilirubin is not yet degraded to sterco- and uro-bilinogen.

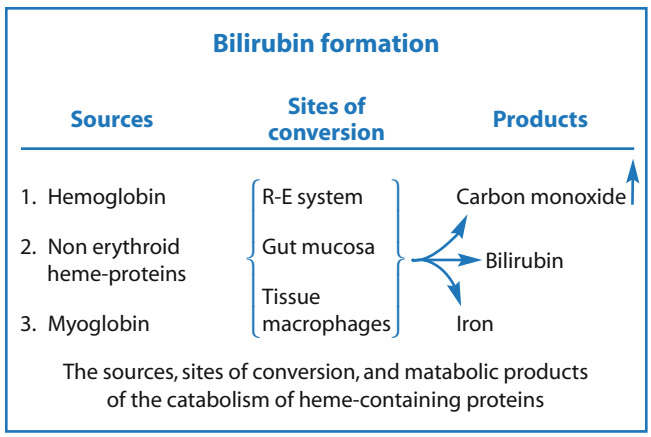

Fig. 81.1 The conversion of heme produces a molecule of carbon monoxide, a molecule of bilirubin and an atom of iron

81.2 Unconjugated Hyperbilirubinemia

Neonatal jaundice is currently defined as hyperbilirubinemia, and bilirubin is 99% unconjugated. In routine laboratory determinations, unconjugated bilirubin is reported as indirect-reacting bilirubin. The only reason for measuring the bilirubin fractions (direct and indirect) concentrations, is the suspicion of cholestatic jaundice, e.g., biliary atresia.

Elevated STB can lead to bilirubin encephalopathy and subsequently to kernicterus with risk of death or permanent

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severe neurodevelopmental disabilities. Jaundice in the absence of a hemolytic disease is frequently and erroneously considered a physiologic or developmental event with very low risk. In fact, in recent years the right emphasis given to breastfeeding has convinced many health personnel that is important to say to mothers "feed the baby on demand, forget the balance and weight gain, and remember that any mother produces the right amount of milk for his baby". Unfortunately, insufficient breastfeeding and starvation increases the activity of heme-oxygenase, the key enzyme in bilirubin production. Thus, in recent times many infants are admitted to hospital after their discharge with a severe dehydration, hypernatremia and hyperbilirubinemia as a consequence of insufficient feeding. Two important papers reported breastfed infants at high risk of kernicterus due to very high hyperbilirubinemia consequent to insufficient nutrition [5, 6]. In the paper of Meisel et al [5] the six infants had a SBC of 39.0–49.7 mg/dL, four had a gestational age of 37 weeks, one of 38 and another of 39 weeks. All had a negative direct Coombs test. They were treated with exchange transfusion but, unfortunately, all resulted as being affected by kernicterus. Three of the four neonates reported by Hansen [6] had a gestational age of 36 weeks while the fourth was born at 40 weeks, but their SBC ranged from 32.1 to 36.3. Three of them were only treated with intensive phototherapy and formula feeding ad libitum while the fourth received an exchange transfusion when SBC was already decreased to approximately 23 mg/dL. All the infants had a normal follow-up.

81.3 Bilirubine Conjugation Genetics

Recently, new research has contributed in clarifying the reason why some healthy infants have a severe jaundice without evident signs of hemolysis, and other infants who are breastfed have a prolonged neonatal jaundice, the so called breast milk jaundice. An important study of Kaplan et al [7] has shown that white neonates with a polymorphism of the gene encoding UGT, in which an additional TA base pair is inserted in TATAA box of the gene promoter (Gilbert's syndrome), could develop hyperbilirubinemia. Affected individuals who are homozygous for the variant promoter, and have 7 TA repeats—$(TA)_7TAA$ (7/7) instead of the more usual 6 repeats—$(TA)_6TAA$ (6/6), have a higher serum total bilirubin (STB) concentration, an increased blood carboxyhemoglobin corrected for inspired CO (COHbc), index of heme catabolism and bilirubin production, a decreased total conjugated bilirubin (TCB) showing a decrease efficacy of enzyme system UDP-Glucuronosyltransferase 1A1 and an increased production/ conjugation rate (COHbc/(TCB/STB [%]), represented by bilirubin production divided by conjugation. In synthesis, newborns with Gilbert's syndrome not only have a diminished enzyme activity which results from decreased expression of normally structured UGT, but also an increased hemolysis which exacerbates neonatal hyperbilirubinemia. This condition is also important because, in the white population, it is frequently associated with prolonged hyperbilirubinemia associated with breastfeeding; after cessation of breastfeeding, the infant's bilirubin level became normal [7]. In the oriental population, a mutation of the UGT 1A1 is responsible of Gilbert's syndrome and prolonged unconjugated hyperbilirubinemia associated with breast milk. The most frequent missense mutation is a G → A transition at nucleotide 211. Arginine replaces glycine at position 71 of the corresponding protein product (G71R) [8].

Identification of genetic mutations of bilirubin conjugation requires a full genetic consultation and laboratory analysis. A detailed genetic work-up should include details of ethnic background, risk factors, previous infants with jaundice, either in the neonatal period or later. This is particularly important because studies have shown that Gilbert's syndrome is a determining factor for the development of neonatal hyperbilirubinemia due to ABO incompatibility [9], and also in G-6-PD deficiency (Mediterranean mutation) [10]. Moreover, Maruo et al, studying Japanese hyperbilirubinemic infants with no obvious cause for their jaundice, found the allele frequency of a mutated UGT 1A1 (G71R) more than double than in controls [8]. Both hetero- and homozygosity for the G71R mutation were associated with hyperbilirubinemia [8]. It is possible that the high incidence of hyperbilirubinemia in Japanese newborns may be a function of the frequency of the G71R mutation in that population. In addition, a case of kernicterus in a breast-fed infant with hereditary spherocytosis and Gilbert's syndrome has been recently reported [11].

Crigler-Najjar (C-N) syndromes are very rare. However, while type 1 C-N is rarely undetected by neonatologists, C-N type 2 is more insidious. In fact, jaundice is very often confused with non-hemolytic jaundice and infants with C-N type 2 could be discharged after phototherapy without any short-term follow-up. Infants often return to the hospital with intense jaundice and signs of bilirubin encephalopathy. C-N type 1 presents with very high hyperbilirubinemia during the first few days of life, and generally phototherapy only transitorily reduces TSB below 20 mg/dL [12]. The treatment with Sn-proto- or mesoporphyrin, heme-oxygenase inhibitors, has been attempted [13, 14] but it is only capable of reducing the time spent under the lamps. However, this treatment could be useful during diseases or starvation when SBC increases over 20–25 mg/dL. By now, the only treatment that could give to these infants a normal life is liver transplant.

81.4 Physiological Neonatal Jaundice

The term "physiological jaundice" is no longer generally used because most neonatologists prefer to describe the level of TSB in relation to time and to treat infants according to a graph of TSB plotted against the baby's age in hours.

Risk zones for near-term (35–37 weeks of gestational age) and term (38–42 weeks of gestational age) newborns have been prepared according to the percentile tracks based on the hour-specific serum bilirubin values [15]. On the age-specific nomogram, the zone >95th percentile was labeled as high risk, and that <5th percentile was labeled as low risk. Serum total bilirubin values between the 5th and 30th, 30th and 60th, and 60th and 95th percentiles were designated as being in the low-intermediate, intermediate, and high-intermediate risk zones, respectively. The 5th and 95th percentiles on the nomogram had the highest sensitivity (100%) and specificity (98.2%), respectively, in predicting the subsequent development for term newborns in the approach to management of hyperbilirubinemia. Infants of 35–37 weeks' gestation had significantly lower birth weights, significantly higher serum total bilirubin levels on days 5 and 7, and were 2.4 times more likely to develop significant hyperbilirubinemia than those of 38–42 weeks' gestation [15]. In near-term newborns of 35–37 weeks' (245–265 days') gestation, the decision to diagnose and treat significant hyperbilirubinemia should be made on the basis of risk status (percentile distribution of the serum bilirubin values on postnatal age) rather than using birthweight-based thresholds. A nomogram constructed from daily serum bilirubin values of each population can be used in assessing the age (hour)-specific jaundice risk (high, intermediate, or low) of each near-term newborn [15].

What is the outcome in a population of healthy term and near-term infants with TSB ≥19 mg/dL (≥325 μmol/L)? To answer this question Jangaard et al [16] have prospectively studied 56,019 infants at ≥35 weeks of gestation who were born between January 1 1994 and December 31 2000 in Nova Scotia, Canada. They did not find cases of kernicterus and no significant differences in rates of cerebral palsy, deafness, or visual abnormalities were observed between infants with hyperbilirubinemia (≥325 μmol/L) and infants with less severe or no hyperbilirubinemia. However, at the follow-up associations with developmental delay, attention-deficit disorder, and autism were observed in the group of hyperbilirubinemic infants [16]. These associations require additional studies to ascertain whether a causal relationship exists.

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82

Pathologic Unconjugated Hyperbilirubinemia, Isoimmunization, Abnormalities of Red Cells and Infections

Michael Kaplan, Ronald J. Wong and David K. Stevenson

82.1 Introduction

Unconjugated hyperbilirubinemia in the newborn after birth is a normal phenomenon, transitional in nature, and harmless. It may be protective for the human neonate exposed to increased levels of oxygen and light in the environment outside the womb before antioxidant enzymatic defenses are fully upregulated. The phenomenon is called "physiologic neonatal jaundice" and the contributing biochemistry has been described in the preceding chapter (see Chapter 81).

Physiologic neonatal jaundice implies benignity, but also a causation that is developmental in nature. That is, newborn infants have normally increased bilirubin production (two–three times the adult on a body weight basis) [1, 2] because of their relatively increased red cell mass responsive to their fetal state and shortened red blood cell lifespan, combined with transitionally impaired uptake and conjugation. This imbalance between bilirubin production and bilirubin elimination is the major determinant of transitional hyperbilirubinemia after birth.

Although pathologic unconjugated hyperbilirubinemia could be defined on the basis of exacerbation of increased bilirubin production or impairment of conjugation, the diagnosis would not even be considered if the pattern of transitional hyperbilirubinemia did not deviate from the normal one. For example, an infant might have markedly increased bilirubin production, but better than usual elimination and therefore, not deviate from the usual course of hyperbilirubinemia. Conceivably, it would also be possible to have greater impairment of uptake or conjugation, but have counter-balancing lower bilirubin production, for example, because of anemia, so that the trajectory of bilirubin levels would not deviate from the expected course. Thus, pathologic unconjugated hyperbilirubinemia is probably best defined epidemiologically, using a nomogram [3], which defines the normal trajectory of hour-specific bilirubin levels, even defining risk zones that reflect the likelihood that a particular infant tracking in that zone might deviate enough from the general population to warrant further diagnostic workup or treatment. Notably, increased bilirubin production is a contributing cause of all kinds of neonatal jaundice. Moreover, all neonates have temporarily impaired elimination of bilirubin. Disorders of hepatic uptake, conjugation, and intrahepatic circulation contribute to the pathologic exacerbation of transitional neonatal jaundice and are discussed in the following chapter (see Chapter 83).

Treatment of pathologic jaundice is also discussed separately. It depends less on the cause (although there may be some consideration of risk in this regard) and more on the extent to which an individual infant has deviated from the normal transitional pattern of hyperbilirubinemia and is at more risk for injury because of the absolute level, binding capacity, and clinical circumstances that might alter binding affinity of albumin for bilirubin.

Thus, pathologic unconjugated hyperbilirubinemia is defined as an hour-specific bilirubin exceeding the 95th percentile for a population-based house-specific bilirubin nomogram, appropriate for the particular ethnic and cultural circumstances [3]. Because all neonates have relatively impaired elimination of the pigment after birth, one of the most common causes of pathologic unconjugated hyperbilirubinemia is increased bilirubin production from any cause. Such causes are the focus of this chapter, and include hemolysis resulting from isoimmunization, abnormalities of red cells and infections, but also many other conditions. Some of these other conditions are quite common, such as closed-space bleeding, most often encountered by the pediatrician in the form a cephalohematoma or bruising. Depending upon the volume of extravascular blood, bilirubin production can be increased to a rate comparable to that observed in hemolytic conditions. Thus, such infants may have a transitional trajectory of bilirubin levels in one of the higher risk zones of the

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hour-specific nomogram or even cross zones depending upon their conjugating capacity and the magnitude of the increase in bilirubin production. Premature infants also have increased production relative to the full term infant, perhaps because of even shorter red cell life spans. The latter is mitigated by less of an increase in the red cell mass among infants born at earlier gestation. Nonetheless, the more mature and larger of the premature infants are likely to deviate from the full-term hour-specific nomogram because of an exaggerated imbalance between increased bilirubin production and impaired elimination [4].

Finally, the infant of a diabetic mother has increased bilirubin production as a major contributing cause to their propensity for pathologic unconjugated hyperbilirubinemia [5, 6]. This increased bilirubin production may be the result of polycythemia, but often reflects ineffective erythropoiesis in the presence of a normal hemoglobin or hematocrit level. The likelihood of a pathologic trajectory of bilirubin levels in the transitional period after birth is further exacerbated by impaired elimination in these infants. This latter compounding factor was revealed by higher bilirubin levels in infants of diabetic mothers compared to infants of normal mothers while controlling for bilirubin production on a body weight basis.

The most important phenomenon contributing to pathologic unconjugated hyperbilirubinemia is hemolysis from any cause. The rapid destruction of red cells in the circulation with the consequent degradation of heme is the predominant source of bilirubin in most pathologic unconjugated hyperbilirubinemia requiring diagnostic workup and treatment. Hemolysis can occur from a variety of specific causes, including isoimmunization (Rh disease, ABO heterospecificity, and other immune globulin mediated hemolytic conditions); red cell enzyme deficiencies (glucose-6-phosphate dehydrogenase [G6PD] deficiency, hexokinase deficiency, and other less common enzymatic deficiencies); and red cell membrane defects (hereditary spherocytosis, elliptocytosis, and other less common red cell membrane defects) [7].

Finally, there are now recognized polymorphisms of the HO-1 (heme oxygenase-1) promoter involving GT expansions [8]. An increase in the number of GT expansions in the promoter can alter the expression of HO-1 and lead to clinically important decreases in bilirubin production [9, 10]. This may have other biological consequences, including risk for vascular disease, and among pregnant women, idiopathic recurrent miscarriages, intrauterine growth restriction, and preeclampsia [8, 11].

What is important to understand in the context of pathologic unconjugated hyperbilirubinemia is the fact that there may be native variation in bilirubin production rates. In the context of impaired elimination in the transitional period after birth or in conditions which further limit uptake or conjugation, these variations might contribute to pathologic unconjugated hyperbilirubinemia without other apparent environmental or acquired exigencies [12–15].

82.2 Hemolytic Conditions

82.2.1 Increased Risk Associated with Hemolysis

Neonates with hemolytic disease appear to be at a higher risk of developing bilirubin induced-brain damage compared with those without hemolysis. Indeed, the first link to be established between increasing bilirubin levels and the risk of kernicterus was in newborns with hemolytic disease due to Rh isoimmunization [16]. Subsequent reports suggested that the incidence of kernicterus in hyperbilirubinemic newborns with hemolytic disease may be higher than that in counterparts without evidence of hemolytic disease, as reviewed by Newman and Maisels [17]. In a survey of the literature up to 1983, Watchko and Oski [18] reinforced the concept that hyperbilirubinemia among neonates without hemolytic disease was less dangerous with regard to the development of kernicterus than in cases where hemolysis was present. A similar stand was taken by Newman and Maisels [19] several years later. However, there is little data on which to base this view. A study shedding some light on this question was performed by Ozmert et al [20]. In 102 children aged 8–13 years, who had been treated for indirect hyperbilirubinemia ranging from 17–48 mg/dL, a positive direct Coombs test, presumed to identify ongoing hemolysis, was associated with lower IQ scores and a higher incidence of neurologic abnormalities. They also found, in these same children, that the incidence of detected neurologic abnormalities increased proportionately to the time of exposure to high bilirubin levels. Similarly, Nilsen et al [21] found that of 55 Norwegian males born in the early 1960s who had neonatal hyperbilirubinemia, those who had a positive Coombs test and hyperbilirubinemia for greater than 5 days had significantly lower IQ scores than average.

Although there is to date no evidence demonstrating higher levels of unbound bilirubin in hemolyzing neonates, it is still generally accepted that hemolysis is a potential factor increasing the risk for bilirubin-related brain damage. While a total serum (or plasma) bilirubin (TSB) concentration of 20–24 mg/dL (342–410 μmol/L) may be associated with kernicterus in a neonate with Rh isoimmunization, a healthy, term non-hemolyzing infant will rarely be endangered by TSB concentrations in this range. Hemolysis due to direct Coombs positive Rh and ABO immunization, other isoimmunizations and G6PD deficiency may therefore pose an increased threat to an otherwise healthy newborn. The Subcommittee on Hyperbilirubinemia of the American Academy of Pediatrics (AAP) includes jaundice developing within the first 24 hours, blood group incompatibility with a positive direct antiglobulin test (DAT) (also known as the Coombs test), other known hemolytic disease including G6PD deficiency, all associated with increased hemolysis, as major risk factors for the development of severe hyperbilirubinemia [22]. The Subcommittee recommends initiating phototherapy or performing exchange transfusions at lower levels of TSB in neonates

with hemolytic conditions than in non-hemolyzing counterparts. However, one should not be lulled into a sense of false security that a hyperbilirubinemic, but non-hemolyzing newborn, will not develop kernicterus. Cases of kernicterus have been reported in which there was no evidence of hemolysis [23]. Crigler-Najjar syndrome, a condition not associated with increased hemolysis, is frequently complicated by bilirubin-induced neurologic dysfunction (BIND).

Unfortunately, there is currently no bedside technique available to determine the presence or absence of increased heme catabolism. Red blood cell (RBC) indices, useful as indicators of hemolysis in older children or adults, are not reliable for this purpose in newborns [24]. Hemolysis, while ongoing, may not be readily determinable. For example, in a multinational, multicenter study of 1370 newborns, the level of corrected end-tidal carbon monoxide (ETCOc), an index of heme catabolism, in 92 of 120 (76%) neonates who were hyperbilirubinemic was greater than the mean value for the cohort [25]. In a male cohort of G6PD-normal, otherwise healthy African American neonates born to non-smoking mothers, ETCOc values were significantly higher among hyperbilirubinemic neonates than among those who did not develop hyperbilirubinemia [26]. Also using ETCOc, Maisels and Kring [27] demonstrated that, before hospital discharge, most infants with TSB levels >75th percentile [3] produced significantly more bilirubin than those with lower bilirubin levels. Because bedside ETCOc testing is no longer available, and blood typing, DAT determination, and G6PD screening are not universally performed, it is possible that an infant not actually classified as having a hemolytic condition, may, in fact, be actively hemolyzing. The potentially increased risk for hyperbilirubinemia and kernicterus may therefore go unrecognized.

Hemolytic conditions in the newborn are generally divided into 2 major etiologic groups: immune and non-immune. The former group includes Rh isoimmunization, although today Rh disease is largely preventable by antenatal and postnatal Rh_oD globulin (of which the best known commercial preparation is Rh_oGAM) administration. Rh disease will, nevertheless, be discussed in some detail, because much of our knowledge of the pathophysiology of kernicterus derives from studies of babies with this condition. ABO immune disease (mother blood group O, baby group A or B) is the most common immune condition currently encountered. Of the non-immune causes, G6PD deficiency is by far the most important, and is highly associated with the development of extreme hyperbilirubinemia and kernicterus.

82.3 Immune Hemolytic Disease

82.3.1 Rh Isoimmune Hemolytic Disease

Rh disease in pregnancy is highly associated with both intrauterine hemolysis as well as severe hemolytic disease following delivery. Untreated, the condition can lead to intrauterine anemia and severe hydrops fetalis, with rapid postnatal evolvement of hyperbilirubinemia with the potential of kernicterus.

82.3.1.1 Background: the Immunization Process

The Rh group of antigens comprises the C, D, and E pairs of antigens. While each of these antigens may result in isoimmunization, anti-D isoimmunization is the most common and has the greatest degree of clinical significance. There is some effect of racial distribution of Rh negativity. In Caucasian populations about 13–15% of individuals are Rh-negative. About half of that number is encountered in African Americans, while Rh negativity is very rare in individuals of Asian background. The incidence of Rh isoimmunization is infrequent, and is reported to be 6.8 cases per 1000 live births [28].

The immunization process may begin if an Rh-negative woman, usually D-negative, is exposed to a D antigen. This may occur by antepartum or intrapartum transplacental fetomaternal transfusion of fetal RBCs containing a D antigen. Similar transfusion of Rh-positive RBCs may occur during abortion, blood administration, or procedures including amniocentesis, chorionic villus sampling, or fetal blood sampling. The mother's immune system may respond by forming anti-D IgG antibodies. These then cross the placenta and adhere to the fetal RBCs containing the D antigen. The antigen-antibody response leads to hemolysis and anemia. Because the mother's immune system becomes primed, the immune response may become more severe and more rapid with progressive pregnancies. In cases of prolonged and severe anemia, bone marrow stimulation may result in increased numbers of circulating immature RBCs (erythroblastosis) and even extramedullary hematopoiesis with hepatosplenomegaly. Fetal hydrops, including generalized tissue edema and pleural, pericardial and peritoneal effusions, may result from hypoproteinemia, tissue hypoxia, and capillary leak, combined with congestive cardiac failure due to anemia, and venous congestions due to poor myocardial function with diminished cardiac output [29].

82.3.1.2 Management of the Pregnancy

Hydrops fetalis is associated with a very high mortality rate and major efforts should be made to prevent this occurrence. The mainstay of the management of a pregnancy complicated by Rh isoimmunization includes active surveillance of the fetus to detect fetal anemia. Once the fetus does become anemic, the decision to perform an intrauterine transfusion or to deliver the infant will depend on the gestational age: the potential complications of delivering a premature infant will have to be weighed against those involved in performing an intrauterine transfusion. In the recent past, amniocentesis was the primary method of fetal monitoring [30]. The degree of hemolysis was assessed by determinations of the amount of

bilirubin in the amniotic fluid by measuring the deviation from linearity at 450 nm, the wavelength at which bilirubin absorbs light. The measurements were divided into 3 zones plotted on the Liley chart. Readings in Zone III were indicative of severe hemolysis with a high likelihood of fetal death. The Liley chart was improved upon by the Queenan curve [31]. This regimen has been largely replaced in the last few years by a combination of advancing ultrasonographic techniques and developing genetic technologies. Because this condition is now rare, and therapeutic technologies are advancing at a rapid rate, Rh-immunized women and their fetuses should be managed in a tertiary center capable of adequately managing the fetus. As the neonatologist not actively involved in this field may be unfamiliar with many of these recent advances, an outline of current management of affected pregnancies follows.

A general principle is that all pregnant women should have an antibody screen performed early in pregnancy. If a woman is RhD-negative and there is no evidence of anti-D alloimmunization, rhesus immune globulin should be administered at 28 weeks gestation [29]. By doing so, the incidence of antenatal immunization will be reduced to 0.1% [32]. The globulin should also be administered following spontaneous or elective abortion, amniocentesis, chorionic villus sampling, or fetal blood sampling. Within 72 hours of delivery, another dose should be administered, should the woman have delivered an RhD-positive infant.

The RhD gene has been localized to the short arm of chromosome 1 [33]. Approximately 55% of individuals will be heterozygous at the RHD locus, in which case only 50% of their fetuses will be RhD-positive. Because an RhD-negative fetus requires no further testing, it is important to assess the chance of an at risk fetus having a D antigen. Gene frequency tables, combined with the history of RhD-positive or -negative infants fathered by any individual have been used to estimate the likelihood of the father being heterozygous. Recent advances in DNA technology, however, allow for accurate determination of whether the father is heterozygous or homozygous for the RHD gene [34]. Should the father be heterozygous, steps should be taken to determine the Rh type of the fetus. This can be done accurately by retrieving fetal DNA via amniocentesis [35]. Amniocentesis for this purpose may be replaced by free fetal DNA techniques, which allow the presence of fetal RHD in the maternal plasma to be detected [34]. Further advances in this field may enable fetal Rh typing to be performed noninvasively in the future.

Determination of the maternal anti-D titer is an important step in the monitoring of an RhD-sensitized woman. A critical titer is that associated with a high risk of severe hydrops. The critical titer varies from center to center, but a titer ranging from 8–32 is usually used [29].

Doppler assessment of the blood flow velocity in the fetus' middle cerebral artery (MCA) is replacing amniocentesis as a means of detecting fetal anemia. In an anemic fetus, the blood velocity may increase due to decreased viscosity and increased cardiac output. In one study, a value of more than 1.5 multiples of the median identified all cases of moderate to severe anemia [36]. In a recent study comparing diagnostic amniocentesis with MCA flow, Doppler measurements improved on optical density determinations by 9% [37]. Should fetal anemia be detected by either Doppler or amniocentesis, fetal blood sampling may be obtained (cordocentesis) to determine hematocrit, direct antibody titer, fetal blood type, reticulocyte count, and total bilirubin values. Intravascular intrauterine transfusion may be considered if the hematocrit value is less than 30%, however, should the pregnancy have reached 35 weeks gestation or more, the advantages of induction of delivery may outweigh the dangers of intrauterine transfusions. Repeated intrauterine transfusion may cause fetal bone marrow suppression, and in a repeatedly transfused fetus, the RBC mass at the time of delivery may be comprised almost entirely of donor cells. In this situation exchange transfusion may be unnecessary, although "top-up" transfusions for anemia may be required [38]. In capable hands, the outcome of intrauterine transfusion should be good. In the Netherlands, the survival rate in 254 treated fetuses was 89% [39].

82.3.1.3 Postnatal Management of the Newborn

Management of a hydropic, severely anemic neonate, especially if complicated by respiratory and other problems of prematurity, is a major neonatal challenge requiring a degree of expertise available only at a tertiary center. The infant may be generally swollen because of soft tissue edema, and in addition may have large collections of fluid in the pleural, pericardial, and peritoneal spaces. Intubation may be difficult because of the soft tissue swelling. Ventilation may be difficult, and the need for emergency drainage of the fluid collections, sometimes in the delivery or operating room, may add to the challenge. Anoxic myocardial damage may necessitate use of inotropes, and respiratory ventilatory support may have to be complemented by surfactant and nitric oxide (NO) administration. High frequency ventilation may be necessary. Metabolic acidosis should be corrected [40].

Because the circulatory system may be overloaded, and myocardial function poor, it may be preferable to correct the anemia by performing a partial exchange transfusion, rather than by simple blood transfusion. Following insertion of an umbilical venous or arterial catheter, small amounts of anemic blood should be removed and replaced with donor blood in the form of packed cells. This procedure may be titrated until the desired hematocrit value is obtained.

In the fetus, most bilirubin formed from increased heme catabolism will be eliminated via the placenta. Severe intrauterine hyperbilirubinemia is therefore not usually not a problem. Once delivered, however, the placenta no longer participates in the bilirubin elimination process, and continued hemolysis combined with immature conjugation and excretion may precipitate a rapid rise in TSB values with the potential, if untreated, of causing extreme hyperbilirubinemia.

Once the baby has been stabilized, attention should be paid to preventing this from occurring, and rectifying this situation should it occur. This is achieved by instituting intense phototherapy as soon as possible, closely monitoring TSB levels, and performing an exchange transfusion in cases where the TSB continues to increase despite intensive phototherapy.

Some studies have demonstrated that intravenous immune globulin (IVIG) administration may be effective in preventing or limiting the number of exchange transfusions in Rh disease [41]. A recent Cochrane report suggested that more information, based on well designed studies, are needed before IVIG can be routinely recommended for isoimmune hemolysis [42]. Nevertheless, the recent AAP guideline recommends the use of IVIG in order to prevent exchange transfusion in cases of failing phototherapy [22]. In general, the principles of the AAP guideline should be adopted to guide the management of the hyperbilirubinemia. The reader is referred to Chapter 84 for further details on the treatment of hyperbilirubinemic newborns.

Very few studies have reported the long-term neurodevelopmental outcome of fetuses treated with intrauterine transfusion. Overall, the results to date are encouraging, although future long-term follow-up studies will be necessary to fully evaluate the current therapeutic modalities offered to the fetuses and newborn infants of Rh-immunized pregnancies [40]. Fetal exposure to moderately increased bilirubin levels for prolonged periods of time may result in sensorineural hearing loss because of the effect of bilirubin on the developing auditory neural system [43].

82.3.2 ABO Immune Disease

82.3.2.1 Background: Blood Group Incompatibility and DAT Positivity

Now that the occurrence of Rh isoimmunization has been reduced by anti-D immune prophylaxis, ABO incompatibility has become the single most frequent cause of immune hemolytic disease causing jaundice in the neonate. Despite its increased frequency, the clinical picture, though, is usually milder than that of Rh disease.

ABO blood group heterospecificity refers to the situation in which the mother has O blood and the baby has blood group A or B. This setup is present in approximately 12% of pregnancies. In some instances, women with blood group O have a high titer of naturally occurring anti-A or anti B antibodies. In contrast to Rh isoimmunization, in which immune sensitization occurs progressively with subsequent pregnancies, high titers of anti-A or anti-B antibodies can sometimes be found in blood group O women before their first pregnancy or even in girls [44]. Unlike blood group A or B individuals, in whom their respective anti-B or anti-A antibodies are IgM molecules, and therefore unable to cross the placenta in significant quantities, the respective antibodies of blood group O people are predominantly IgG molecules. Being smaller than IgM, IgG molecules may cross the placenta and attach to the corresponding fetal RBCs, provided that these cells have the A or B antigen. This isoimmunization process may cause hemolysis commencing in utero. Extravascular hemolysis of the IgG-coated RBCs is probably mediated by Fc-receptor-bearing cells within the reticuloendothelial system. Prior to delivery, there is little danger of severe hyperbilirubinemia and the immune process is usually not sufficiently severe to cause hydrops. Babies may sometimes be born moderately anemic, and there is a potential danger of hyperbilirubinemia in the first days following delivery.

The hallmark of isoimmunization is a positive DAT. The test is termed direct if the antiglobulin is detected on the RBCs. An indirect test refers to the antibody being detected in the serum. About one-third of blood group A or B neonates born to a blood group O mother will have a positive DAT [45]. Measurements of endogenous formation of CO, reflective of heme catabolism, have demonstrated an increased rate of heme catabolism in hyperbilirubinemic DAT-positive, ABO-incompatible neonates compared with controls [2, 46, 47]. In a multicenter, international study of bilirubin production using ETCOc determinations, DAT positive babies (not necessarily hyperbilirubinemic) had significantly higher ETCOc values than the total population studied [25]. While hyperbilirubinemic, DAT positive babies had even higher ETCOc values than the general DAT positive population, these were not significantly higher than hyperbilirubinemic babies of other categories.

Not all DAT positive neonates will develop severe hyperbilirubinemia. In one study, although a positive DAT was predictive of an elevated TSB value, only 20% of DAT positive neonates actually developed TSB values >12.8 mg/dL (218 μmol/L), and even fewer had severe jaundice [45]. In another study, only 19.6% of DAT positive, ABO-incompatible infants required phototherapy [48]. Despite this apparent clinical mildness, the authors occasionally encounter newborns with severe hyperbilirubinemia of early onset, occasionally warranting exchange transfusion. To complete this clinical spectrum, kernicterus has been described in association with ABO hemolytic disease [49, 50].

The reasons for absence of clinical disease in the majority of DAT-positive newborns may include the paucity of A and B antigenic sites on neonatal RBCs compared with adult RBCs, or weak expression of these antigens in neonates. Non-RBC A or B antigenic sites may exist and bind with transplacentally-acquired antibody without affecting the RBC. ABO blood group incompatibility with a negative DAT, not usually predictive of hemolysis or hyperbilirubinemia, may sometimes cause early and rapidly progressing jaundice, reminiscent of DAT-positive, hemolytic disease.

ABO heterospecificity with a positive DAT does not necessarily indicate ABO hemolytic disease as many ABO incompatible, DAT positive neonates have no evidence of

ongoing hemolysis and do not develop early jaundice or hyperbilirubinemia. In order to support the diagnosis of ABO hemolytic disease, some or all of the following criteria are necessary:

- Indirect hyperbilirubinemia, especially during the first 24 hours of life
- Mother with blood group O, baby with blood group A or B
- Spherocytosis on blood smear
- Increased reticulocyte count
- Evidence of hemolysis based on increased endogenous production of CO. Unfortunately, a readily available clinical too for determining ETCOc levels is no longer available.

An interaction between DAT negative ABO incompatibility and polymorphism for the (TA)7 sequence in the promoter of the UDP-glucuronosyltransferase 1A1 (UGT1A1) gene, which encodes the corresponding bilirubin conjugating enzyme, has been described. Neonates who were both ABO incompatible as well as homozygous for the variant gene promoter had a significantly higher incidence of TSB ≥ 15.0 mg/dL (257 μmol/L) than counterparts not bearing this combination [51].

82.3.2.2 Clinical Manifestations of ABO Hemolytic Disease

Most blood group A or B neonates born to blood group O mothers will not develop any signs of hemolytic disease. Routine blood group and DAT determination on umbilical cord blood is an option, but not mandatory. It is essential to closely observe any newborn born to a blood group O mother for the development of jaundice. A TSB measurement should be performed at the first sign of visible jaundice. Phototherapy and exchange transfusions are indicated according to the 2004 AAP guidelines [22], using the lower level of TSB intended for newborns with risk factors. IVIG may be helpful in modifying the rate of rise of bilirubin and may be particularly useful in situations in which the TSB is approaching the indications for exchange transfusion, despite a trial of intense phototherapy. It is thought that IVIG blocks the Fc receptors in the reticuloendothelial system, thereby inhibiting hemolysis and limiting bilirubin formation.

82.3.3 Isoimmunization Due to Antibodies Other than RhD

More than 50 RBC antigens may cause hemolytic disease of the newborn [52]. The most important of these with regard to prenatal hemolysis and the need for intrauterine infection include anti-c, anti-Kell, and anti-E [53–55], although others may also, infrequently, be problematic. Alloimmunization due to these autoantibodies can sometimes cause severe hemolytic disease of the fetus requiring prenatal intervention. Fetal surveillance protocols and clinical strategies developed for Rh D alloimmunization are useful in monitoring all alloimmunized pregnancies. Similarly, the postnatal management should be based on the principles outlined in the management of the RhD-immunized newborn. Anti-Kell isoimmunization warrants special mention as fetal anemia often predominates the clinical picture. This may be due to erythropoietic suppression in addition to a hemolytic process [56].

82.4 Non-Immune Hemolytic Disease

82.4.1 Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

G6PD deficiency is one of the most common enzyme deficiencies known and is estimated to affect hundreds of millions of people worldwide [57, 58]. Immigration patterns have transformed this condition from one limited to its indigenous distribution, including areas in south Europe, Africa, the Middle East, and Asia, to one which may now be encountered virtually in any corner of the globe. G6PD deficiency is a major factor in the pathogenesis of hyperbilirubinemia and is highly associated with extreme hyperbilirubinemia and kernicterus [59, 60]. The condition is overrepresented in a recent series of neonates with extreme hyperbilirubinemia and bilirubin encephalopathy, relative to the overall frequency of this condition in the United States, Canada, United Kingdom and Ireland [49, 50, 61]. For example, in the US-based Pilot Kernicterus Registry [49], over 20% of reported neonates had G6PD deficiency, whereas the overall frequency of G6PD deficiency in that country is estimated at less than 3% [58]. Because G6PD deficiency has major public health implications as far as the neonate is concerned, it will be discussed below in some detail.

82.4.1.1 Function of G6PD

G6PD plays a major part in stabilization of the RBC membrane. The enzyme catalyzes the first step in the hexose monophosphate pathway, oxidizing glucose-6-phosphate to 6-phosphogluconolactone, thereby reducing NAPD to NADPH. NADPH is essential for the regeneration of reduced glutathione from oxidized glutathione, a substance that plays an integral part in the body's antioxidative mechanisms. The pathway is also instrumental in stimulating catalase, another important antioxidant. In the absence of G6PD, NADPH will not become available, reduced glutathione will not be regenerated, and cells may be rendered susceptible to oxidative stress. The RBC is especially vulnerable to the G6PD deficiency state, as, unlike other body cells, no alternative source of NADPH is available in that cell. Oxidative membrane damage incurred may manifest as hemolysis [58].

82.4.1.2 Genetics of G6PD Deficiency

G6PD deficiency is an X-linked condition. Males may therefore be normal hemizygotes or deficient hemizygotes. Females may be either normal or deficient homozygotes, or heterozygotes. The most common mutations are G6PD A^-, found in Africa and southern Europe and in African Americans. G6PD Mediterranean, regarded as a more severe type than G6PD A^-, is encountered in Mediterranean countries, the Middle East and India. Another variant encountered primarily in Asia is G6PD Canton. Many other mutations have been documented [57].

82.4.1.3 G6PD Deficiency and Hemolysis

Most G6PD-deficient individuals will lead perfectly normal lives and for the most part be unaware of their inherited condition. However, G6PD deficiency is notoriously associated, in children and adults, with severe hemolytic episodes, associated with jaundice and anemia, which may occur following exposure to a hemolytic trigger. Classically, these episodes often occur following ingestion of or contact with the fava bean (favism). Beutler [57] has emphasized the role of infections in the pathogenesis of acute hemolysis.

The most extreme form of G6PD deficiency associated hemolytic jaundice in neonates is typified by acute and unpredictable onset of jaundice. Some identifiable substances which may trigger hemolysis include naphthalene used to store clothes, herbal medicines, henna applications, or menthol-containing umbilical potions. Frequently, however, no identifiable trigger can be found. Extreme jaundice may develop suddenly and with no prior warning, and the TSB concentration may increase exponentially to dangerous levels. G6PD deficiency may therefore by the one reason that kernicterus may not be completely preventable. The hemolysis may be severe and exchange transfusion may be the only recourse.

Frequently hematologic indices typical of hemolysis in older children and adults, including falling hemoglobin and hematocrit values and increasing reticulocyte count may be absent, despite a clinical picture of hemolysis. However, studies of endogenous CO formation, reflective of the rate of heme catabolism, have demonstrated an important role of increased hemolysis in association with this condition [62, 63]. Slusher et al [63] for example, demonstrated significantly higher levels of carboxyhemoglobin (COHb) in Nigerian G6PD-deficient neonates who developed kernicterus, compared with neonates who were hyperbilirubinemic, but did not develop signs of kernicterus.

More frequently and less life-threatening, G6PD-deficient neonates may have a more moderate form of jaundice. The incidence of this form of jaundice occurs at a rate several-fold over that of controls. The jaundice usually responds to phototherapy, although exchange transfusion may also be necessary. These infants have a low-grade hemolysis, which cannot be implicated as the primary icterogenic factor [64, 65]. Diminished bilirubin conjugation has been shown to be of major importance in the pathogenesis of the jaundice [66]. A intriguing interaction has been noted between G6PD deficiency and the non-coding area $(TA)_7$ promoter polymorphism in the gene encoding the bilirubin conjugating enzyme, UGT1A1 [67]. This polymorphism, also known as UGT1A1*28, is associated with Gilbert's syndrome. The incidence of hyperbilirubinemia, defined as a TSB ≥15.0 mg/dL (257 μmol/L), increased in a stepwise, dose-dependent fashion, in G6PD-deficient neonates who were either heterozygous or homozygous for the polymorphism. This effect was not seen in the G6PD-normal control group. Furthermore, G6PD deficiency alone, in the absence of the promoter polymorphism, did not increase the incidence of hyperbilirubinemia over and above that of G6PD-normal counterparts. In contrast, in Asians, in whom the (TA)7 polymorphism is rare, a similar interaction was noticed between G6PD deficiency and coding area mutations of the UGT1A1 gene [68].

Unlike the acute hemolytic form of jaundice, this milder form of jaundice can be predicted by predischarge TSB testing. Neonates who had a predischarge TSB concentration below the 50th percentile on the bilirubin nomogram [3] had a very small likelihood of subsequent hyperbilirubinemia. As the predischarge TSB increased above the 50th percentile, however, the chance of subsequent hyperbilirubinemia increased significantly [69].

82.4.1.4 Testing for G6PD Deficiency

Many qualitative or quantitative screening tests are available which should accurately determine the hemizygous state in males or the homozygous state in females. However, being an X-linked condition, a large section of the female population may comprise heterozygotes. Because of X-inactivation, heterozygotes have two RBC populations: G6PD-deficient, and G6PD-normal. Non-random X-inactivation may result in unequal ratios of normal and enzyme deficient RBCs. Heterozygotes may, as a result, have either a normal, intermediate or deficient phenotype rendering the heterozygote state difficult to determine using standard biochemical tests [70]. It was previously thought that heterozygotes had sufficient enzyme activity to protect them from the dangers of G6PD deficiency [58]. However, more recent reports suggest that heterozygotes may not be without risk [71–73]. Females of high risk groups should be closely followed-up according to the development of jaundice, a normal reading screen notwithstanding. Also, biochemical tests may give a falsely normal result if performed during an acute hemolytic episode, as older RBCs may be destroyed, leaving younger cells, with higher enzyme activity, intact [74]. In such cases, G6PD testing should be performed several weeks after the acute hemolysis has subsided. An alternate method is to analyze DNA for the specific suspected mutation.

Some countries have introduced neonatal screening for G6PD deficiency combined with parental education. There is

little data demonstrating whether screening will actually be instrumental in reducing the incidence of kernicterus. Parents of screened newborns and their medical attendants should, however, be made more aware of the condition by the screening process. Parental education should lead to avoidance of potentially offending substances. Hopefully, knowledge that an infant is G6PD-deficient should speed the process of evaluation and treatment of a jaundiced neonate.

The treatment of neonatal hyperbilirubinemia associated with G6PD deficiency should follow the guideline of the AAP [22] for neonates with hemolytic risk factors. In the event an infant presents with neurological signs commensurate with bilirubin encephalopathy, exchange transfusion should be immediately performed.

82.4.2 Pyruvate Kinase Deficiency

Pyruvate kinase catalyzes the conversion of phosphoenolpyruvate to pyruvate and the formation of adenosine triphosphate (ATP) from adenosine diphosphate in the Embden-Meyerhof pathway. Pyruvate kinase deficiency, a condition inherited in an autosomal recessive manner [75], results in a lack of ATP, an important source of energy for RBC metabolism [76]. As a result, in the newborn period, anemia, reticulocytosis, and severe jaundice may ensue. Exchange transfusion may be required. This RBC enzyme defect is much less frequently encountered than G6PD deficiency. Four isozymes are encoded by 2 genes, among which 180 mutations have been described [77]. Diagnosis is determined by enzyme assay, which should be performed in cases of hemolysis and hyperbilirubinemia not associated with a positive DAT or spherocytosis. Molecular studies may also confirm the diagnosis.

82.4.3 Hereditary Spherocytosis

Of the hereditary RBC membrane defects which may lead to acute hemolysis and hyperbilirubinemia in the newborn, hereditary spherocytosis is probably the most common [78, 79]. Hereditary spherocytosis is also one of the few causes of hyperbilirubinemia which does not resolve in the neonatal period. This condition may be inherited both in an autosomal dominant and recessive fashion, and frequently there may be a history of acute hyperbilirubinemia in a sibling or a parent. It is thought that protein deficiencies in the RBC membrane, including ankyrin, band 3, α-spectrin, β-spectrin, and protein 4.2, leave microscopic patches of the lipid bilayer inner surface bare of proteins, at which points microvesiculization occurs. These osmotically fragile RBCs are trapped in the spleen, the microvesicles aspirated by macrophages, and the cell destroyed. Hemolysis may result in jaundice, anemia, and splenomegaly. The diagnosis can be made microscopically by demonstrating spherocytes in the peripheral blood smear, with confirmation by the osmotic fragility test. The latter test may be especially important in differentiating babies with hereditary spherocytosis from those with DAT positive ABO isoimmunization, a condition that may also result in microspherocytosis. Mutations of at least 5 genes encoding the above-mentioned proteins have been recognized.

Hereditary spherocytosis is frequently associated with neonatal hyperbilirubinemia. Of 178 affected Italian, term, predominantly breastfed newborns, 112 (63%) developed neonatal hyperbilirubinemia requiring phototherapy. The incidence of hyperbilirubinemia was even higher in those who also had a genetic variation in the promoter of the bilirubin UGT1A1 gene, similar to that described in G6PD deficiency [78]. Kernicterus has been described [10]. Treatment comprises intense phototherapy with resort to exchange transfusion in cases unresponsive to light therapy. Splenectomy may become necessary later during childhood in order to control the anemia resulting from ongoing hemolysis.

82.4.4 Hereditary Elliptocytosis, Pyropoikilocytosis, Ovalocytosis, and Stomatocytosis

These are rare conditions affecting the erythrocyte membrane. The diagnosis may be made by microscopic examination of the peripheral blood smear. Hemolysis may occur in the neonatal period and result in anemia and hyperbilirubinemia.

82.4.5 Infection

Infection is often associated with pathologic unconjugated hyperbilirubinemia. The presence of infection also creates increased risk for injury caused by bilirubin, probably related to alterations in binding of bilirubin to albumin, but also through other mechanisms that may not be well-defined, related to the inflammatory process [80]. The likelihood of injury caused by bilirubin is increased for a given level of bilirubin in the presence of infection. Moreover, an infant is more likely to deviate from the normal trajectory of bilirubin levels because of increased bilirubin production in the presence of infection. The cause of this increased production may be hemolysis, but also upregulation of heme oxygenase (heat shock protein 32), which may respond to oxidative stress associated with the infected state.

82.5 Summary

In summary, pathologic unconjugated hyperbilirubinemia is best defined epidemiologically. Although some pathologic unconjugated hyperbilirubinemia can be understood in terms

of primary problems with uptake and conjugation, most pathologic unconjugated hyperbilirubinemia encountered by the pediatrician after birth and requiring treatment is associated with increased bilirubin production, often further exacerbated by environmental or acquired factors. Thus, a treatment strategy designed to modulate bilirubin production remains rational and potentially important, if a drug with the appropriate safety profile and efficacy is approved. Meanwhile, phototherapy is the mainstay of treatment and is reviewed in Chapter 84.

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Kernicterus, Bilirubin Induced Neurological Dysfunction and New Treatments for Unconjugated Hyperbilirubinemia

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83.1 Introduction

In the 19th century it was already known that unconjugated hyperbilirubinemia could potentially harm the central nervous system of jaundiced newborn infants. Yellow staining of deep brain nuclei in jaundiced infants was first reported in 1847. The term kernicterus (in German, *kern* = nucleus; in Greek, *ikterus* = yellow) was first denoted in 1903 to describe the pathological findings of this specific yellow staining pattern [1]. Nowadays, kernicterus is not only used to describe the pathological findings, but also to describe the clinical findings of acute and/or chronic bilirubin encephalopathy in jaundiced infants [2, 3]. Although acute kernicterus is an unambiguous clinical disorder in severely jaundiced newborn infants with the possibility of permanent sequelae, subtle forms of bilirubin encephalopathy referred to as bilirubin-induced neurological dysfunction, also known as BIND have evolved more recently [4]. This chapter aims to describe the pathophysiology of bilirubin neurotoxicity, its clinical spectrum and diagnostic tools. Novel treatment modalities to prevent infants from developing severe unconjugated hyperbilirubinemia and bilirubin neurotoxicity will be highlighted.

83.2 Pathophysiology – Risk Factors

The risk of kernicterus and BIND may be in part determined by the concentration of Total Serum Bilirubin (TSB), which in neonates consists almost exclusively of unconjugated bilirubin (UCB), but is primarily determined by the concentration of non-albumin bound free bilirubin (B_f). B_f can easily pass the blood-brain barrier, and may better reflect the bilirubin load distributed in the brain [5]. Several cellular mechanisms of the brain protect the brain against bilirubin accumulation. One of these protective regulating mechanisms is UCB export from the brain to the blood by multidrug resistance P-glycoprotein 1 (MDR1) and most importantly by the multidrug resistance-associated protein 1 (MRP1) [5, 6]. Data from *in vitro* and *in vivo* experiments have suggested that B_f, and not TSB, is the principal determinant of bilirubin neurotoxicity [7–9]. A number of pathophysiological factors are related to the variable clinical spectrum of bilirubin neurotoxicity. First, several mechanisms including necrosis and apoptosis are involved in bilirubin-induced neuronal damage, resulting in specific types of cellular damage and dysfunction [5, 6, 10]. Second, neural susceptibility to bilirubin neurotoxicity is not comparable in all cell types of the brain, neurons being more susceptible than glia cells with the exact mechanisms involved remaining speculative [5, 6, 10]. Third, the amount of UCB that enters the brain is dependent not only on the B_f concentration, but also on the intactness of the blood brain barrier. The B_f concentration in plasma is determined by the concentration of unconjugated bilirubin bound to albumin, at a specific plasma pH, in relation to the affinity of bilirubin to bind albumin. Conditions associated with low albumin concentrations, or with displacement of bilirubin from albumin (e.g., by sulfonamides or free fatty acids) increase B_f concentrations. Conditions that decrease intactness of the blood-brain barrier (e.g., hyperosmolality, hypercapnia, asphyxia, prematurity, infection, and sepsis) can result in a net increase of UCB uptake in the brain [4].

Multiple other factors (i.e., cerebral blood flow, vascular permeability, cellular efflux pumps and cellular recovery capacity) may affect the development of neurotoxicity at any given B_f concentration [6]. Some of these factors are incorporated as risk factors in management guidelines of jaundiced infants. Clinical evidence of most of these risk factors is limited. Most factors are based on anecdotal clinical evidence or theoretical and experimental animal data. Although imperfect, these factors are used by many clinicians to determine TSB thresholds before starting treatment [2, 3, 11–15]. The risk factors that increase the susceptibility for bilirubin neurotoxicity

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 83.1 Risk factors used in addition to TSB to assess the risk of bilirubin neurotoxicity in the management of jaundiced infants

Risk factors
– Acidosis
– Asphyxia
– Hemolysis
– Hypothermia
– Hypoalbuminemia or less albumin available to bind bilirubin
– Intracranial hemorrhage
– Low birth weight
– Meningitis
– Prematurity
– Sepsis

and risk factors that predispose to develop hyperbilirubinemia are shown in Table 83.1. Risk assessment of bilirubin neurotoxicity for each individual newborn infant involves more than measurement of the TSB concentration, and appears to be a dynamic process affected by continuously changing individual conditions. Unfortunately, B_f cannot be routinely measured so that concentration of TSB and the currently used risk factors are the principle parameters that determine treatment thresholds.

83.3 Clinical Symptoms

The clinical spectrum of signs of bilirubin neurotoxicity relates to the bilirubin-induced damage to specific brain areas. Specific brain stem nuclei (auditory, vestibular and oculomotor), cerebellar Purkinje cells, basal ganglia (i.e., globus pallidus and subthalamus) and the hippocampus are particularly vulnerable to bilirubin neurotoxicity [6, 16]. In the initial phase, acute bilirubin encephalopathy is characterized by lethargy, hypotonia and poor sucking. In the second or intermediate phase, most commonly within 2–3 days after the initial phase, hypertonia (retrocollis, opistotonus), discomfort and irritability dominate. The infant may develop fever, a typical high-pitched cry, and seizures, which may alternate with drowsiness and hypotonia. Exchange transfusion at this stage might reverse the central nervous system damage in some cases. When left untreated, this second phase progresses into a more advanced stage characterized by pronounced retrocollis-opisthotonus, shrill cry, apnea, fever, sometimes seizures, deep stupor to coma and even death [3, 17]. Choreoathethosis, vertical gaze paralysis, auditory dysfunction, and dental dysplasia (Perlstein's tetrad) and motor delay are the most commonly described permanent neurologic sequelae in infants who survive the acute phase of kernicterus. Intelligence is usually normal in these infants [5, 17]. Less severe hyperbilirubinemia can result in subtle bilirubin encephalopathy referred to as "bilirubin-induced neurological dysfunction" (BIND) or as "bilirubin-associated neurological dysfunction" (BAND) [18]. Subtle permanent bilirubin encephalopathy can present with auditory dysfunction and/or mild neurologic abnormalities such as mild impairment in neurologic and/or cognitive performance [4, 19]. Involvement of the auditory nervous system dysfunction may result in sensorineuronal hearing loss or deafness. Alternatively, auditory dysfunction referred to as auditory neuropathy (AN) or auditory dys-synchrony (AD) may occur. AN/AD is defined as a normal neurophysiological test of the inner ear, i.e., normal cochlear microphonic responses and otoacoustic emissions, but an abnormal or absent auditory brainstem response (ABR) resulting in abnormal sound processing. AN/AD is clinically characterized by problems in sound localization and speech discrimination. Hearing loss is not present per se and a normal audiogram is often seen [4].

Several studies have evaluated effects of moderate and severe degees of hyperbilirubinemia with respect to neurodevelopmental outcome in later childhood [20]. Considering the differences in incidence and severity of neurologic dysfunction in infants with a comparable severity of neonatal hyperbilirubinemia, it seems impossible to predict outcome by TSB levels in jaundiced newborn infants. In general, preterm infants and infants suffering hemolytic disease seem to have an increased risk of neurologic sequelae of neonatal hyperbilirubinemia [20]. Isolated movement disorders such as athetosis or distonia are sometimes seen and in a retrospective analysis of extremely low birth weight infants a significant association between peak concentrations of TSB during the first two weeks of life and neurodevelopmental impairment was found [4, 17, 19, 21, 22].

83.4 Epidemiology

In the past century, kernicterus was almost exclusively seen in the context of high concentrations of TSB related to Rh hemolytic disease. When Rh disease became rare due to the introduction of Rh-immune globulin, and phototherapy appeared an effective treatment for unconjugated hyperbilirubinemia, the incidence of kernicterus decreased. Subsequently, treatment criteria were liberalized. As a combined result of the more liberal treatment criteria, a trend towards earlier hospital discharge (before the maximal TSB concentration is reached), and higher survival rate of preterm infants, kernicterus remains a serious threat for jaundiced newborn infants. Nowadays, the exact incidence of kernicterus and BIND is unknown. This is, at least in part, related to differences in the definition of severe hyperbilirubinemia and in methods of assessing neurodevelopmental outcome among studies. In a prospective study investigating severe hyperbilirubinemia (defined as maximum TSB >510 μmol/L) of the newborn in the UK, the incidence of bilirubin encephalopathy was ~ 1 case per 100,000 live births [23]. It is estimated that 1–3 per 100,000 live births are at risk of developing kernicterus when untreated and that 5–10% of infants surviving severe hyperbilirubinemia suffer permanent sequelae [6, 20].

83.5 Diagnosis

Current management guidelines for jaundiced infants are actually based on the total serum bilirubin (TSB) concentrations. Because exact neurotoxic TSB concentrations are unknown and risk factors for imminent bilirubin neurotoxicity are not evidence based, other diagnostic tools, in addition to TSB, may be valuable to detect imminent BIND in jaundiced newborn infants (Table 83.2).

To evaluate the risk of BIND, the history of the infant is important. Information about the gestational age, physical examination and the presence of risk factors for bilirubin neurotoxicity as well as the duration of the hyperbilirubinemic period, the presence of acute symptoms and whether the child has previously been treated or not is crucial data to diagnose BIND at a later age [24].

Although preliminary clinical series and *in vitro* data point to a correlation between B_f and BIND, the difficulty of clinically measuring B_f has prevented its introduction in clinical practice. Alternatively, the Bilirubin/Albumin ratio (B/A ratio) could be used as a surrogate parameter to indicate B_f concentrations [25, 26]. However, due to the presence of bilirubin displacers, i.e., drugs that interfere with the bilirubin-albumin binding, B_f may reach values much higher than suggested by the calculated B/A ratio. In addition, the individual variability in the binding affinity of the albumin for bilirubin should also be taken into account. Alternatively, other plasma constituents such as apolipoproteins may bind UCB, and decrease B_f. Consequently, the B/A ratio seems an imperfect surrogate to estimate B_f. It needs to be outlined however, that B_f may also be an imperfect predictor of bilirubin neurotoxicity, since many factors affect the development of bilirubin neurotoxicity at any given B_f level.

The theoretical considerations and clinical evidence for the concept that additional use of the B/A ratio, i.e., next to TSB, in jaundiced premature infants might improve the prediction of BIND has been recently reviewed. Although no prospective clinical trials exist, it is suggested that the additional use of the B/A ratio may be valuable in evaluating jaundiced premature infants [27–31]. A randomized controlled trial investigating the additional use of the B/A ratio in the treatment of hyperbilirubinemia in preterm infants (ISRCTN74465643) is underway and will hopefully provide the answer to this important question.

Table 83.2 Diagnostic tools to evaluate the severity of hyperbilirubinemia and/or risk of imminent bilirubin neurotoxicity

Diagnostic tool
Anamnestic
– History of hyperbilirubinemic period
– Treatment for neonatal hyperbilirubinemia
– Gestational age
– Risk factors present in hyperbilirubinemic period
– Symptoms of kernicterus during hyperbilirubinemic period
– Abnormal physical examination
Total serum bilirubin concentration
Free bilirubin concentration
Bilirubin/albumin ratio
Auditory brainstem response
Magnetic resonance imaging
BIND score

The possibility that biochemical markers, i.e., use of Tau and S100B protein concentrations, are useful in the diagnosis of bilirubin-induced neurotoxicity has been studied in hyperbilirubinemic newborn infants [32]. Tau is a microtubule-associated structural protein of central nervous system neurons. S100B protein, a neurotrophic factor, is synthesized in astroglial cells in the central nervous system and Schwann cells. Previous data showed increased Tau and S100B protein concentration in plasma and/or cerebrospinal fluid in patients with cerebral injury (e.g., hypoxia and trauma). In a prospective study of 92 hyperbilirubinemic non-asphyxiated infants, Tau and S100B protein concentration was positively correlated with TSB concentration. Tau and S100B concentration increased at TSB concentration above 327 μmol/L, as observed in 46 infants. At lower TSB concentrations, protein concentrations remained unchanged. Above a TSB concentration of 327 μmol/L, clinical symptoms of bilirubin encephalopathy (i.e., auditory neuropathy, minor neurologic dysfunction and electroencephalographic abnormalities) were present in 22 of 46 of the infants. Compared to TSB concentrations, measurement of Tau and S100B protein concentration did not improve sensitivity or specificity for any of the described clinical symptoms. The use of Tau and S100B protein concentration in the assessment of BIND is not recommended.

The neural auditory pathway is very susceptible to bilirubin toxicity putatively resulting in sensorineurinal hearing loss or auditory neuropathy also known as auditory dys-synchrony. A frequently used, non-invasive and very sensitive tool to determine bilirubin neurotoxicity, is the Auditory Brainstem Response (ABR), which allows for determination of electrophysiological activity of the neural auditory pathway. The ABR consists of a sequence of positive waves (numbered I–V) representing the auditory pathway from inner ear to brainstem. Wave I and II represent the peripheral auditory nerve and waves III–V represent the activity in the auditory centers at the brain stem level of the pathway (cochlear nucleus and lateral lemniscus, respectively) [33]. Bilirubin-induced ABR changes mainly involve waves III and V and may progress from reversible increased interwave latencies to the loss of wave amplitude. ABR changes can be transient, but may also progress into permanent wave changes or even loss of any recognizable wave [7, 27]. A bedside method to evaluate the intactness of the auditory pathway is the Automated Auditory Brainstem Response (AABR) with an ALGO hearing screening system (Natus Medical, San Carlos, CA, USA). AABR measurements are simplified ABR measurements and able to identify infants with abnormal cochlear or auditory function. A pass or refer result is shown on the ALGO machine for each ear of the infant. In an observational study of

191 patients of variable birth weight (406–4727 g) and variable gestational age (24–42 weeks), an abnormal ALGO result (bi-or unilateral refer) was associated with increased B_f concentrations and B_f/TSB ratios, but not with TSB concentrations alone [34].

Another tool to identify acute and chronic bilirubin neurotoxicity in jaundiced infants is Magnetic Resonance Imaging (MRI) [18]. MRI changes include bilateral hyperintensity of globus pallidus on T1-weighted scans in the early phase (first 3 weeks of life) and most commonly subtle but persistent pallidal hyperintensity, suggestive of permanent gliosis, on late T2-weighted scans (with disappearance of hyperintensity on T1-weighted scans). The subthalamic nucleus and hippocampus are affected less frequently (approximately 40% and 5% of reported cases, respectively). Bilateral damage of the globus pallidus and the subthalamic nucleus are specific signs of bilirubin neurotoxicity, which sometimes can also be visualized with ultrasonography, and is a key in the differentiation between hypoxic-ischemic encephalopathy or metabolic disorders, which predominantly affect the thalamus [30]. The additional value of MR spectroscopy to MRI is not completely clear, but acute changes in cerebral metabolism have been shown in bilirubin encephalopathy [35].

In 1999, a clinical scoring system was developed to evaluate the risk of exposure to unconjugated hyperbilirubinemia. Clinical signs of bilirubin encephalopathy include mental status, muscle tone and cry of the infant. Dependent on the level of abnormality, 0-3 points are obtained for each clinical sign, resulting in an overall score between 0 and 9, representing no toxicity or advanced toxicity, respectively. Validation of this scoring system is in progress [36, 37].

83.6 Treatment

To identify newborns at risk for severe hyperbilirubinemia and to prevent bilirubin neurotoxicity, guidelines have been developed for the management of jaundiced infants. The American Academy of Pediatrics' Subcommittee on Hyperbilirubinemia adapted a guideline for hyperbilirubinemic infants of 35 or more weeks of gestation in 2004 [3].

Currently, this guideline is adapted by many countries worldwide for the management of jaundiced "near-term" and term newborn infants. However, international guidelines for preterm infants are lacking and rather local or national guidelines for preterm infants are used, such as the consensus-based guideline on hyperbilirubinemia for preterm infants of 35 or less weeks of gestational age in The Netherlands (www.neonatologiestudies.nl/main/richtlijnen) [38].

Few prospective studies have analyzed effects of different TSB-thresholds on long-term outcome (Table 83.3). In a group of 95 low birth weight infants, hyperbilirubinemia was treated either with prophylactic phototherapy (starting 12 hours after birth, n = 46) or conservative phototherapy (starting at fixed TSB threshold concentrations of 150 μmol/L, n = 49). The maximum TSB concentrations were comparable in both groups, but in a subgroup of extreme low birth weight infants (ELBW, < 1000 g), the maximum TSB concentrations were significantly higher in the conservative group (171 μmol/L versus 139 μmol/L, conservative versus prophylactic treatment) with a concomitant higher incidence of cerebral palsy. However, since the study was not originally powered for this outcome, it failed to demonstrate significant differences in long-term neurodevelopmental outcome [39]. Another randomized controlled trial assigned 1974 ELBW preterm infants to a prophylactic phototherapy group (started immediately postnatal) or a conservative phototherapy group (based on predefined TSB threshold concentrations: 137 μmol/L for infants weighing 501–750 g and 171 μmol/L for infants weighing 751–1000 g). The primary outcome of this study was a combination of neurodevelopmental impairment (defined as blindness, severe hearing loss, moderate of severe cerebral palsy, or a score below 70 on the mental or psychomotor developmental index of the Bayley Scale of Infant Development II) or death. In this

Table 83.3 Long-term outcome of randomized controlled trials on TSB thresholds

Author/Year	Population	Phototherapy		Long-term outcome
		Prophylactic	Conservative	
Morris, 2008 [40]	ELBW infants (<1000 g) n = 1974	Direct postnatally	TSB threshold 137 μmol/L (501–750 g) or 171 μmol/L (751–1000 g)	No significant difference for the combination of death or neurological impairment. Prophylactic phototherapy reduced the risk of neurodevelopmental impairment (RR = 0.86, 95% CI: 0.74–0.99) Non-significant higher mortality among infants of 501–750 g (RR = 1.13, 95% CI: 0.96–1.34)
Jangaard, 2007 [39]	LBW infants (<1500 g) n = 95	12 hours postnatally	TSB threshold 150 μmol/L	Non-significant increase in cerebral palsy and death in a subgroup of ELBW infants (<1000 g) in the conservative group

ELBW extreme low birth weight, i.e., birth weight less than 1000 g; *LBW* low birth weight, i.e., birth weight less than 1500 g, *RR* relative risk, *CI* confidence intervals, 17.1 μmol/L = 1 mg/dL bilirubin.

study, maximum TSB concentrations were significantly higher in the conservative group: 168 μmol/L versus 120 μmol/L in the prophylactic group. Prophylactic phototherapy significantly reduced the risk of neurodevelopmental impairment (relative risk [95% confidence intervals]: 0.86 [0.74–0.99]). This benefit of prophylactic phototherapy seems questionable for infants with a birth weight of 501–750 g, because in this group a non-significant trend of increased mortality was found [40].

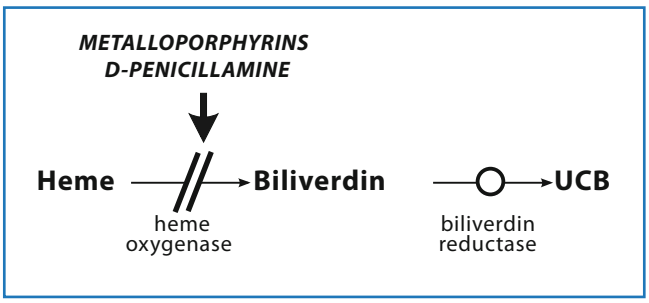

Fig. 83.1 Inhibitors of heme oxygenase effectively decrease the conversion of heme into biliverdin, the first essential step in bilirubin production. Reproduced from [43], with permission

83.7 New Treatments for Unconjugated Hyperbilirubinemia

Conventional treatment for severe unconjugated hyperbilirubinemia consists of phototherapy and exchange transfusion. Although phototherapy is considered effective and safe, it does not always prevent toxic accumulation of bilirubin in neonates. Long-term phototherapy, as required by Crigler-Najjar patients, may take up to 16 hours per day and becomes less effective with age. In spite of this intensive treatment regimen, approximately 30% of the patients with Crigler-Najjar disease type I develop mild to severe brain damage. In addition, patients still die resulting from complications related to the disease [41]. Exchange transfusion should be considered if phototherapy fails to decrease plasma bilirubin below toxic levels. This "rescue-treatment" however, is associated with a significant morbidity in sick newborns, and even mortality has been reported. These considerations have prompted investigators to develop alternative treatments for unconjugated hyperbilirubinemia [42]. Most of these treatments are still in an experimental phase. Nevertheless, it is highly conceivable that some of them will find their way into clinical practice in the foreseeable future.

83.7.1 Treatments that Decrease the Production of Bilirubin

Treatments that decrease the production of bilirubin could actually prevent unconjugated hyperbilirubinemia, which would be a more rational strategy compared with removing bilirubin that is already accumulated in the body (see Chapter 84). Bilirubin is produced in the macrophages of the reticuloendothelial system. These macophages contain two essential enzymes: heme oxygenase (HO) and biliverdin reductase. Heme oxygenase converts heme, the source of bilirubin, into blue-green biliverdin. Biliverdin reductase then converts biliverdin into bilirubin. Consequently, bilirubin production can be decreased by agents that inhibit HO, and/or biliverdin reductase (Fig. 83.1). Several agents have been developed to inhibit these enzymes such as the metalloporphyrins [43, 44]. Metalloporphyrins compete with heme for HO binding sites resulting in a competitive inhibition of HO and in a decreased conversion of heme into biliverdin. Currently, tin-mesoporphyrin is the most evaluated metalloporphyrin in humans. In the 8 clinical trials so far conducted, it was demonstrated that this agent mitigates unconjugated hyperbilirubinemia due to ABO incompatibility and prematurity. Also, the use of tin-mesoporhyrin replaced the need for phototherapy in glucose-6-phosphate dehydrogenase deficient newborns. Tin-mesoporphyrin thus seems a highly promising new treatment strategy for unconjugated hyperbilirubinemia. Currently, however, metalloporhyrins cannot be recommended for routine treatment of unconjugated hyperbilirubinemia due to insufficient evidence regarding the long-term safety of these agents.

D-penicillamine, a chelating agent that is used in the treatment of Wilson's disease, is another HO-inhibitor (Fig. 83.1) [43]. The use of this agent decreased the number of exchange transfusions in infants with ABO-hemolytic disease, but its efficacy has only been evaluated in a single clinical trial. Curiously, inhibitors of biliverdin reductase have never been explored, most likely because the accumulation of biliverdin in the blood would produce green babies.

83.7.2 Treatments that Increase the Hepatic Clearance of Biliribin

Almost all bilirubin is excreted via the bile, and many therapies aim to enhance this excretory pathway by targeting the hepatic clearance of the bilirubin. Newly produced bilirubin enters the microcirculation of the liver from where it is extracted by the hepatocytes. In the hepatocyte, bilirubin is firstly bound to cytosolic ligandin, which prevents its diffusion back into the blood. The enzyme UDP-glucuronosyltransferase 1A1 (UGT1A1), which resides in the endoplasmatic reticulum of the hepatocytes, subsequently conjugates bilirubin with one or two glucuronic acid groups.

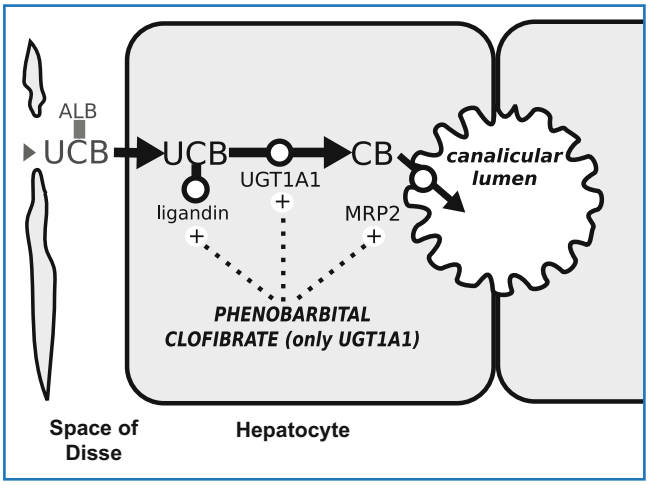

Fig. 83.2 Phenobarbital and clofibrate increase the hepatic clearance of bilirubin. *Alb* albumin, *UCB* unconjugated bilirubin, *UGT1A1* UDP-glucuronosyltransferase1A1. Reproduced from [43], with permission

This step increases the water solubility of the molecule and allows the now conjugated bilirubin to be excreted into the bile via the canalicular transporter MRP2. Several therapies target these 3 steps in bilirubin catabolism (Fig. 83.2). Clofibrate, a lipid-lowering drug, increases UGT1A1 activity (Fig. 83.2). This drug mitigated neonatal unconjugated hyperbilirubinemia in several clinical trials, and decreased the need for phototherapy if applied as add-on treatment. However, long-term clofibrate use is associated with an overall increase in non-cardiovascular mortality. Although short-term clofibrate treatment has not been shown to have serious adverse effects, its safety issues must be addressed before it could be considered for clinical use.

The most well-known agent that enhances hepatic bilirubin clearance is phenobarbital. This antiepileptic agent enhances ligandin, UGT1A1, and MRP2 activity (Fig. 83.2). Since the 1960s, phenobarbital has been used extensively in the treatment of neonatal jaundice. Numerous clinical trials have shown that administering phenobarbital to pregnant women or to newborns mitigates neonatal hyperbilirubinemia and decreases the number of exchange transfusions. Nevertheless, phenobarbital is currently not used as a routine treatment of neonatal jaundice. This is mainly because phototherapy is more effective, decline of TSB concentrations in infants treated with phenobarbital and phototherapy is not faster when compared to phototherapy alone, and because its therapeutic effect is not evident until a few days after administration, in contrast to some of the adverse effects (i.e., sedation) [43]. Phenobarbital is still applied in Crigler-Najjar type II patients, who have a 95% decrease in UDP-glucuronosyltransferase activity. In these patients phenobarbital is able to increase the residual enzyme activity, which effectively counteracts the development of severe unconjugated hyperbilirubinemia. Phenobarbital is not effective in the treatment of Crigler-Najjar type I, however, because these patients lack residual (e.g., inducible) UDP-glucuronosyltransferase activity.

The most effective treatment for Crigler-Najjar disease type I would be to repair or replace the defective UGT1A1 in the liver. Currently, this can only be achieved by a liver transplantation. In 1986 the first successful orthotopic liver transplantation (OLT) in a Crigler-Najjar patient was reported, and several other patients have undergone transplantation since.

Two types of transplantation have been used: OLT in which the patients' own liver is replaced by a donor liver, and auxiliary liver transplantation in which part of the own liver is left in situ and is supported by a donor graft. If successful, a liver transplant completely corrects the underlying metabolic defect, which dramatically improves the quality of life. Liver transplantation, however, remains a high-risk procedure, with a one-year survival between 85% and 90%. Infusion of hepatocytes with an unimpaired UGT1A1 activity into the liver of Crigler-Najjar patients would be an attractive alternative to a liver transplant. This procedure, which is known as hepatocyte transplantation, could partially restore enzyme activity without the many complications that are associated with a liver transplant. So far, 7 Crigler-Najjar type I patients have received this treatment, often with multiple infusions of hepatocytes (for example via the portal vein). Hepatocyte infusion, however, decreased plasma bilirubin levels only for a limited period of 5–6 months and did not eliminate the need for a liver transplant in these patients [45, 46]. Ultimately, gene therapy would be the most elegant method to repair or replace defective UGT1A1 within the hepatocytes of Crigler-Najjar type I patients. The results of gene therapy seem promising in animal models [47]. Currently, however, the results of ongoing clinical trials must be awaited before gene therapy can be applied in Crigler-Najjar patients.

83.7.3 Treatments that Decrease the Enterohepatic Circulation of Bilirubin

After conjugation, bilirubin enters the intestinal lumen via the bile. The intestinal conjugated bilirubin is subsequently mostly hydrolyzed to UCB, which can be reabsorbed into the enterohepatic circulation (Fig. 83.3a). The majority of this reabsorbed UCB, however, spills over into the systemic circulation due to the poor first pass extraction by the liver. Conditions that enhance this enterohepatic circulation contribute to the pathogenesis of unconjugated hyperbilirubinemia. For example, poor feeding in neonates is associated with increased plasma bilirubin levels most likely caused by a delayed gastrointestinal transit, which increases the amount of intestinal UCB available for reabsorption. Indeed, conditions that accelerate the gastrointestinal transit, such as early

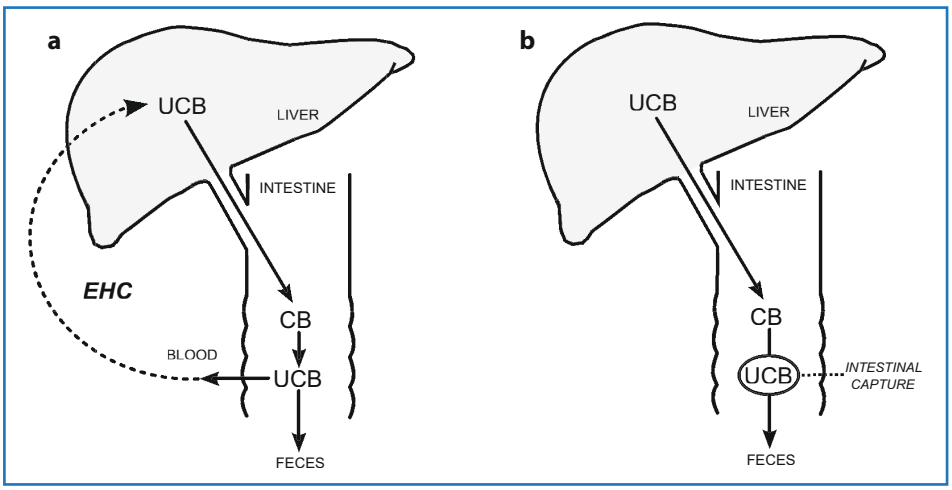

Fig. 83.3 **a** Normally, and especially in conditions that delay the intestinal transit, there is an enterohepatic circulation of unconjugated bilirubin which increases plasma bilirubin levels. **b** Binding unconjugated bilirubin to capture agents in the intestinal lumen may prevent this enterohepatic circulation and thus decrease plasma bilirubin levels. *UCB* unconjugated bilirubin *CB* conjugated bilirubin, *EHC* enterohepatic circulation. Reproduced from [43], with permission

and frequent feedings, seem to lower plasma bilirubin levels in newborn infants [48, 49]. Bilirubin reabsorption could also be prevented by agents that bind and capture the pigment within the intestinal lumen (Fig. 83.3b).

Several capturing strategies have so far been tested. Cholestyramine, a known binder of bile salts, and agar, a gelatinous substance derived form seaweed, decreased plasma bilirubin levels in hyperbilirubinemic rats, but was less effective in neonates. Treatment of neonates with activated charcoal effectively decreased plasma bilirubin levels, but only if it was administered within the first day of life. Charcoal, however, might also capture essential nutrients, which limits its clinical applicability [43]. Oral amorphous calcium phosphate did lower plasma bilirubin levels, but only in Crigler-Najjar type I patients. Calcium phosphate is currently used in a number of Dutch Crigler-Najjar type I patients as an adjunct to phototherapy if plasma unconjugated bilirubin levels become dangerously elevated. Orlistat, a lipase inhibitor, increases the intestinal fat content, which is hypothesized to capture the lipohilic unconjugated bilirubin. Orlistat has been shown to lower plasma bilirubin levels in a trial with Crigler-Najjar patients, but the decrease was considered clinically relevant (i.e., >10%) in only 7 of 16 patients. Zinc salts are also well-known binders of unconjugated bilirubin, and moderately decreased plasma bilirubin levels in patients with Gilbert's syndrome [43]. This inherited condition is characterized by a chronic, mild unconjugated hyperbilirubinemia related to diminished hepatic UGT1A1 expression. Administration of zinc salts, however, may lead to increased zinc levels in plasma, which may limit their clinical use.

83.8 Future Prospective

As indicated above, the molecular events leading to BIND are still partially undefined, as the criteria are not fully described to define BIND when the damage may be successfully treatable. It is therefore necessary to approach this increasingly present damage in a more translational way linking *in vitro* with *in vivo* models. This will hopefully provide hints on an effective prevention of the neurological damage in addition to the established phototherapy. To this end we need to better understand how unconjugated, free bilirubin may enter the cell and how the cells handle this potentially toxic substance. We know that MRP1 may extrude UCB from cells but data obtained *in vitro* suggest that the activity of this ABC transporter is not the only player in reducing UCB cytotoxicity. Intracellular oxidation of UCB may be an additional mechanism that needs to be assessed, as pharmacological inducers of oxidizing pathways are available. Also it is still unknown why UCB accumulates and damages only certain regions of the brain. Understanding this unexplained uneven distribution will unravel the background for regional sensitivity. Collectively these data will hopefully allow the definition of more effective treatments not only for the newborns but also for patients with Crigler-Najjar type I.

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84

Treatment of Hyperbilirubinemia

M. Jeffrey Maisels and Jon F. Watchko

84.1 Introduction

The indications for the treatment of hyperbilirubinemia and the methods used for treatment vary according to the individual circumstances. Thus, treatment of hyperbilirubinemia can be prophylactic, when the purpose is to prevent further increase in the total serum bilirubin (TSB) level, or therapeutic, where the objective is to rapidly decrease a TSB level that is a threat to the infant. In some units, phototherapy is initiated soon after birth in all infants whose birth weight is <1000 g, irrespective of the TSB level. The TSB level can be lowered (or prevented from increasing further) in one of three ways: a) exchange transfusion, which removes bilirubin mechanically; b) phototherapy, which converts bilirubin to products that can bypass the liver's conjugating system and be excreted in the bile or the urine without further metabolism and c) pharmacologic agents that interfere with heme degradation and bilirubin production, accelerate the normal metabolic pathways for bilirubin clearance, or inhibit the enterohepatic circulation of bilirubin.

Table 84.1 Guidelines for the use of phototherapy and exchange transfusion in low birthweight infants based on birth weight

	Total bilirubin level *			
Birth weight	Phototherapy †		Exchange transfusion ‡	
(g)	mg/dL	μmol/L	mg/dL	μmol/L
<1500	5–8	85–140	13–16	220–275
1500–1999	8–12	140–200	16–18	275–300
2000–2499	11–14	190–240	18–20	300–340

Note that these guidelines reflect ranges used in neonatal intensive care units. They cannot take into account all possible situations. Lower bilirubin concentrations should be used for infants who are sick – for example, presence of sepsis, acidosis, hypoalbuminemia – or have hemolytic disease.

* Consider initiating treatment of these levels. Range allows discretion based on clinical conditions or other circumstances. Note that bilirubin levels refer to total serum bilirubin concentrations. Direct reacting or conjugated bilirubin levels should not be subtracted from the total.

† Used at these levels and in therapeutic doses, phototherapy should, with few exceptions, eliminate the need for exchange transfusions.

‡ Levels for exchange transfusion assume that bilirubin continues to rise or remains at these levels despite intensive phototherapy.
From [86], with permission.

84.2 Using Phototherapy and Exchange Transfusion to Prevent Kernicterus and/or Neurodevelopmental Impairment

A selection of recommendations and guidelines for the use of phototherapy and exchange transfusion in term, late preterm and preterm infants are provided in Tables 84.1–84.3 and Figs. 84.1 and 84.2. These recommendations vary, and are based, primarily, on the TSB levels, but also on gestation, birth

Table 84.2 Guidelines for use of phototherapy and exchange transfusion in preterm infants based on gestational age

	Total bilirubin level					
GA	Phototherapy		Exchange transfusion			
			Sick*		Well	
(weeks)	mg/dL	μmol/L	mg/dL	μmol/L	mg/dL	μmol/L
36	14.6	250	17.5	300	20.5	350
32	8.8	150	14.6	250	17.5	300
28	5.8	100	11.7	200	14.6	250
24	4.7	80	8.8	150	11.7	200

* Rhesus disease, perinatal asphyxia, hypoxia, acidosis, hypercapnia.
GA gestational age. Modified from [87].

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Table 84.3 Guidelines according to birth weight for exchange transfusion in low birthweight infants based on total serum bilirubin and bilirubin/albumin ratio (whichever comes first)

	< 1250 g	1250–1499 g	1500–1999 g	2000–2499 g
Standard Risk				
Total bilirubin mg/dL (μmol/L)	13 (222)	15 (257)	17 (291)	18 (308)
B/A ratio mg/g (μmol/μmol)	5.2 (0.61)	6.0 (0.71)	6.8 (0.80)	7.2 (0.85)
*High Risk**				
Total bilirubin mg/dL (μmol/L)	10 (171)	13 (222)	15 (257)	17 (291)
B/A ratio mg/g (μmol/μmol)	4.0 (0.47)	5.2 (0.61)	6.0 (0.71)	6.8 (0.80)

* Risk factors: Apgar < 3 at five minutes; PaO_2 < 40 mmHg ≥ 2 h; pH ≤ 7.15 ≥ 1h; birth weight < 1000g; hemolysis; clinical or central nervous system deterioration; total protein ≤ 4 g/dL or albumin ≤ 2.5 g/dL. B/A ratio, Bilirubin/albumin ratio. From [21], with permission.

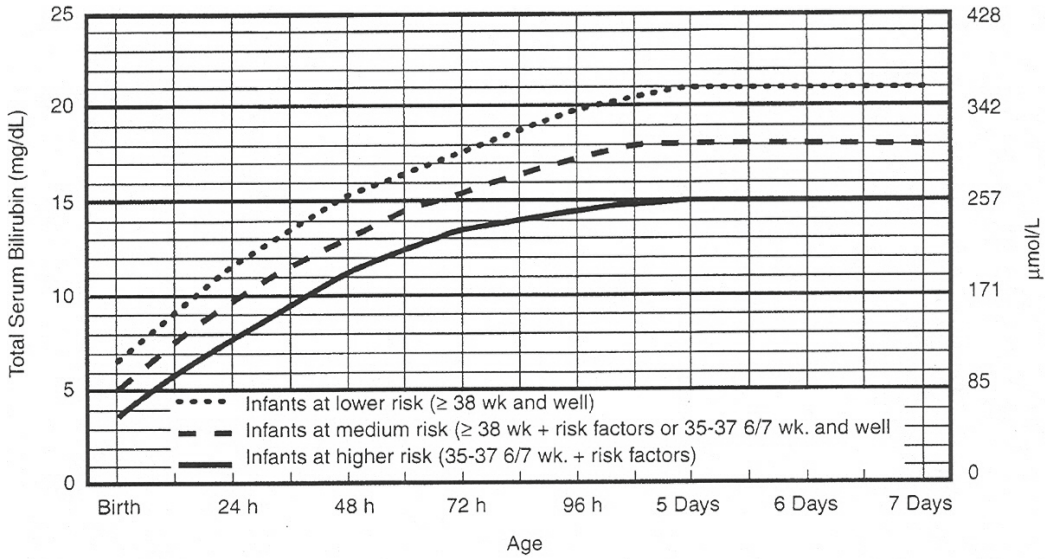

Fig. 84.1 Guidelines for phototherapy in hospitalized infants of 35 or more weeks' gestation. From [22], with permission.
– Use total bilirubin. Do not subtract direct reading or conjugated bilirubin.
– The lines for lower, medium and higher risk refer to risk for neurotoxicity.
– Risk factors for neurotoxicity – isoimmune hemolytic disease. G6PD deficiency, asphyxia, significant lethargy, temperature instability, sepsis, acidosis or albumin < 3.0 g/dL (if measured).
– For well infants 35–37 6/7 wk can adjust TSB levels for intervention around the medium risk line. It is an option to intervene at lower TSB levels for infants closer to 35 wks and at higher TSB levels for those closer to 37 6/7 wk.
– It is an option to provide conventional phototherapy in hospital or at home at TSB levels 2–3 mg/dL (35–50 mmol/L) below those shown but home phototherapy should not be used in any infant with risk factors.

These guidelines refer to the use of intensive phototherapy, which should be used when the TSB exceeds the line indicated for each category. Infants are designated as "higher risk" because of the potential negative effects of the conditions listed on albumin binding of bilirubin [24–26], the blood-brain barrier [82], and the susceptibility of the brain cells to damage by bilirubin [82].

Intensive phototherapy implies irradiance in the blue-green spectrum (wavelengths of approximately 430–490 nm) of at least 30 $\mu W/cm^2$ per nm (measured at the infant's skin directly below the center of the phototherapy unit) and delivered to as much of the infant's surface area as possible. Note that irradiance measured below the center of the light source is much greater than that measured at the periphery. Measurements should be made with a spectroradiometer specified by the manufacturer of the phototherapy system.

If the total serum bilirubin does not decrease or continues to rise in an infant who is receiving intensive phototherapy, this strongly suggests the presence of hemolysis. From [22], with permission.

weight, bilirubin/albumin ratios, and risk factors that increase the risk of bilirubin encephalopathy. Thus intervention at lower TSB levels is recommended in more premature infants and for those in whom risk factors for bilirubin-induced brain damage are present. It would be ideal if these guidelines were the product of evidence-based estimates of when the benefit of these interventions exceeded their risks and costs. Such estimates should come from randomized trials or high quality, systematic observational studies, but these studies are rare [1, 2]. Treatment guidelines must, therefore, rely on relatively

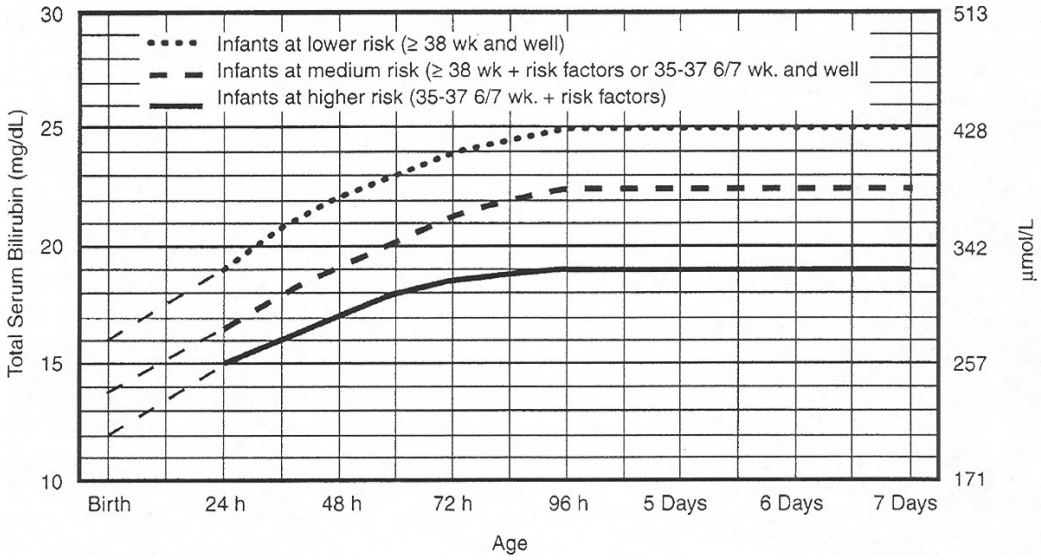

Fig. 84.2 Guidelines for exchange transfusion in infants 35 or more weeks' gestation. From [22], with permission.
- The dashed lines for the first 24 hours indicate uncertainty due to a wide range of clinical circumstances and a range of responses to phototherapy.
- Immediate exchange transfusion is recommended if infant shows signs of acute bilirubin encephalopathy (hypertonia, arching, retrocollis, opisthotonos, fever, high pitched cry) or if TSB is ≥ 5 mg/dL (85 μmol/L) above these lines.
- The lines for lower, medium, and higher risk refer to risk for neurotoxicity.
- Risk factors for neurotoxicity - isoimmune hemolytic disease, G6PD deficiency, asphyxia, significant lethargy, temperature instability, sepsis, acidosis.
- Measure serum albumin and calculate B/A ratio (see legend).
- Use total bilirubin. Do not subtract direct reacting or conjugated bilirubin.
- If infant is well and 35–37 6/7 wk (median risk) can individualize TSB levels for exchange based on actual gestational age.

During birth hospitalization, exchange transfusion is recommended if the TSB rises to these levels despite intensive phototherapy. For readmitted infants, if the TSB level is above the exchange level, repeat TSB measurement every 2–3 hours and consider exchange if the TSB remains above the levels indicated after intensive phototherapy for 6 hours.

The following B/A ratios can be used together with but in not in lieu of the TSB level as an additional factor in determining the need for exchange transfusion. From [22], with permission.

Risk Category	B/A ratio at which exchange transfusion should be considered	
	TSB mg/dL/Alb, g/dL	TSB μmol/L/Alb, μmol/L
Infants ≥ 38 0/7 wk	8.0	0.94
Infants 35 0/7–36 6/7 wk and well or ≥ 38 0/7 wk if higher risk or isoimmune hemolytic disease or G6PD deficiency	7.2	0.84
Infants 35 0/7–37 6/7 wk if higher risk or isoimmune hemolytic disease or G6PD deficiency	6.8	0.80

If the TSB is at or approaching the exchange level, send blood for immediate type and crossmatch. Blood for exchange transfusion is modified whole blood (red cells and plasma) crossmatched against the mother and compatible with the infant [84].

uncertain estimates of risks and benefits as well as the recognition that using a single TSB to predict long-term behavioral and developmental outcomes is not reliable and will lead to conflicting results [1, 2].

Although kernicterus has been reported in term and near-term infants at levels below 25 mg/dL [1], such outcomes are rare, and, if TSB levels meeting or exceeding the American Academy of Pediatrics (AAP) exchange transfusion criteria (Fig. 84.2) are treated promptly, the outcomes are generally benign [3, 4]. Setting thresholds for intervention in the premature neonate is more challenging. Framing of guidelines for the use of phototherapy and exchange transfusion in preterm infants (Tables 84.1–84.3) has been a capricious exercise at best, and one for which no claim of an "evidence-base" can be made [5]. These guidelines are provided by different experts, none of whom would make any claim for the greater validity of one approach versus another [5]. A recent report from the United Kingdom showing a remarkably wide range of treatment thresholds for phototherapy and exchange transfusions in premature neonates, further underscores this uncertainty [6].

Phototherapy is effective in lowering the circulating bilirubin or preventing it from rising further. Its main demonstrated value is that it reduces the need for exchange transfusion although recent evidence suggests that it might also have a role in reducing neurodevelopmental impairment in infants of extremely low birth weight (ELBW, ≤1000 g) [7]. After this chapter went to press, two additional guidelines were published for the treatment of the jaundiced newborn [88, 89].

84.3 Assessing the Risk of Kernicterus and Neurodevelopmental Impairment in Preterm Infants

Although the concept of low bilirubin kernicterus in sick, low birth weight infants has long been recognized, kernicterus continues to be reported in preterm infants at low bilirubin levels and in the absence of acute neurologic signs [8–10]. Recently, kernicterus was documented in 2 preterm infants, 31 and 34 weeks of gestation, neither of whom were acutely ill and whose TSB levels were 13.1 mg/dL (224 μmol/L) and 14.7 mg/dL (251 μmol/L) respectively. These reports renew concerns about low-bilirubin kernicterus in the premature infant [8,9].

Until quite recently, there were no large studies of ELBW infants looking at the association between peak TSB levels and neurodevelopmental outcome. A follow-up of 2575 ELBW infants showed an association between peak serum bilirubin and (a) death or neurodevelopmental impairment (odds ratio [OR] 1.068: 95% CI 1.03–1.11): (b) psychomotor developmental index (PDI) < 70 (OR 1.057: 95% CI 1.00–1.12) and (c) hearing impairment (OR 1.138: 95% CI 1.00–1.30) [11]. Some ELBW neonates have also developed athetoid cerebral palsy, and hearing loss [7], features similar to term infants with kernicterus [9] as well as MRI findings characteristic of chronic bilirubin encephalopathy [9, 12, 13].

84.3.1 Unbound or Free Bilirubin

Recognition that a peak TSB level, by itself, is a rather poor predictor of the likelihood of poor neurodevelopmental outcome or kernicterus, raises the question of whether we should be using measurements of unbound or "free" bilirubin (B_f) or the ratio of bilirubin to albumin (B/A) to predict hyperbilirubinemia risk [14, 15]. Most bilirubin in the circulation is bound to albumin and a relatively small fraction remains unbound. The concentration of B_f is believed to dictate the biologic effects of bilirubin in jaundiced newborns, including its neurotoxicity. Elevations of B_f have been associated with kernicterus in sick, preterm infants [16, 17]. In addition, elevated B_f concentrations are more closely associated than TSB with transient abnormalities in the brainstem auditory evoked potential (BAEP) in both term [18] and preterm [19] infants. Although in one study a B_f level of > 1.0 μg/dL predicted the presence or absence of neurotoxicity in preterm neonates with high sensitivity and specificity [17], there is no agreement about what constitutes the neurotoxic B_f threshold [20] i.e., the threshold at which B_f produces changes in cellular function culminating in permanent cell injury and cell death. In addition, clinical laboratory measurement of B_f is not generally available.

The ratio of bilirubin (mg/dL) to albumin (g/dL) does correlate with measured B_f in newborns [21] and has been used as an approximate surrogate for the measurement of B_f [21] and the AAP has endorsed this approach [22]. A randomized controlled trial in the Netherlands (the BARTrial) is testing the use of the B/A ratio in conjunction with the TSB to determine when phototherapy and/or exchange transfusion should be used and will evaluate neurodevelopmental outcome at 18–24 months corrected age [23]. It must be recognized, however, that albumin binding capacity varies significantly between newborns [21, 24], is impaired in sick infants [25–27] and increases with increasing gestational age [25, 28] and postnatal age [26, 27]. A recent study of VLBW infants from one National Institute of Child Health and Human Development (NICHD) Neonatal Network institution confirmed that bilirubin-binding capacity was lower and B_f higher in unstable versus stable neonates but did not report on longer term neurodevelopmental outcomes [29]. Follow-up in the phototherapy trial of 224 infants born in 1974–1976 with birth weights < 2000 g and evaluated at age 6 years, showed no relation between measures of bilirubin-albumin binding and IQ scores [30]. It will be instructive to see how measures of B_f correlate with short- and long-term outcomes in the NICHD Neonatal Network ELBW cohort and how the bilirubin to albumin ratio correlates with neurodevelopmental outcome in the BARTrial [23].

Crucially important in the measurement of B_f is the bilirubin-albumin binding constant k, a term whose numeric value may actually vary considerably depending on conditions including among other factors, sample dilution, albumin concentration, and the presence of competing compounds [14, 15, 20]. Moreover, the risk of bilirubin encephalopathy is likely not simply a function of the B_f concentration alone or TSB level but a combination of both, i.e., the total amount of bilirubin available (the miscible pool of bilirubin) as well as the tendency of bilirubin to enter the tissue (the B_f concentration) [21].

An additional factor is the susceptibility of the cells of the central nervous system to damage by bilirubin [31, 32]. The B/A ratio can therefore be used together with, but not in lieu of, the TSB level as an additional factor in determining the need for exchange transfusion [21] (Table 84.3, Fig. 84.2). Clarifying and defining clinically germane B_f concentrations, bilirubin to albumin ratios, exposure conditions, and exposure durations, as well as improving, standardizing, and validating B_f measurements are important lines of clinical and translational research.

Table 84.4 Criteria for initiating phototherapy and exchange transfusions in the NICHD Neonatal Research Network Trial [7]

Birth weight	Aggressive management			Conservative management			
	Phototherapy begins	Exchange transfusion		Phototherapy begins		Exchange transfusion	
		mg/dL	μmol/L	mg/dL	μmol/L	mg/dL	μmol/L
501–750 g	ASAP after enrollment	≥13.0	≥222	≥8.0	≥137	≥13.0	≥257
751–1000 g	ASAP after enrollment	≥15.0	≥257	≥10.0	≥171	≥15.0	≥257

Enrollment is expected within the period 12–36 hours after birth, preferably between 12 and 24 hours.

84.3.2 Aggressive Versus Conservative Management of Infants with Birth Weights ≤1000 g

The NICHD Neonatal Research Network conducted a randomized controlled trial of "aggressive *vs* conservative" phototherapy in 1974 infants who were followed to age 18–20 months of corrected age [7]. The protocol for this study is shown in Table 84.4. Compared with conservative phototherapy, aggressive phototherapy did not reduce the primary outcome of death or neurodevelopmental impairment but, in surviving infants, did reduce the rates of (a) neurodevelopmental impairment (relative risk [RR] 0.86: 95% CI 0.74–0.99), (b) hearing loss (RR 0.32: 95% CI 0.15–0.68), mental development index score <70 (RR 0.83: 95% CI 0.71–0.98) and athetosis (RR 0.20: 95% CI 0.04–0.90). The reduction in neurodevelopmental impairment was attributable almost entirely to a decrease in infants with profound impairment in the aggressive phototherapy group (RR 0.68: 95% CI 0.52–0.89). The mean TSB levels were lower in the aggressive group (4.7 ± 1.1 mg/dL [80.4 ± 19 μmol/L]) than the conservative group (6.2 ± 1.5 mg/dL [106 ± 26 μmol/L]) and, although these differences were statistically significant ($p < 0.001$) it is surprising that this small difference was associated with a difference in outcome. On the other hand, the mean TSB levels in the surviving impaired and unimpaired infants were identical (5.4 mg/dL [92 μmol/L]) although the mean peak TSB was marginally higher in the impaired cohort (8.6 ± 2.3 mg/dL [147 ± 39 μmol/L] versus 8.3 ± 2.3 mg/dL [142 ± 39 μmol/L], $p = 0.02$).

It was noteworthy, however, that there was a 5% increase in mortality in infants with birth weights of 501–750 g who received aggressive phototherapy. This was not statistically significant, but a post-hoc Bayesian analysis estimated an 89% probability that aggressive phototherapy increased the rate of death in this subgroup. It is unclear why phototherapy might increase mortality in these tiny infants but it is likely that light penetrates more deeply through the thin, gelatinous skin, reaching the subcutaneous tissues and possibly producing oxidative injury to cell membranes [33].

What should neonatologists do with this information? In many units, phototherapy is initiated in ELBW neonates when their TSB reaches 5 mg/dL (86 μmol/L). As the TSB at the start of phototherapy in the aggressive group was 4.8 mg/dL (82 μmol/L), instituting phototherapy at a TSB of 5 mg/dL (86 μmol/L) will likely have a similar effect on TSB levels as does prophylactic phototherapy initiated in every infant soon after birth. When one combines these data with the previous observations from the NICHD Neonatal Research Network [11], it appears that modest elevations of TSB in these tiny babies are potentially harmful and, when used in a manner similar to that employed in this study, phototherapy could help to reduce long-term neurodevelopmental impairment. From the total population of 1974 infants only 5 (0.25%) required an exchange transfusion.

84.4 Phototherapy

Phototherapy uses visible light energy to change the shape and structure of bilirubin, converting it to molecules that can be excreted even when normal conjugation is deficient. Light provides an infusion of discrete photons of energy that correspond to the individual molecules of a drug in a conventional medication. Absorption of these photons by bilirubin molecules in the skin leads to a therapeutic effect in much the same way as binding of drug molecules to a receptor has a desired effect. Fig. 84.3 provides a detailed description of how phototherapy works. The relative contributions of the various reactions (Fig. 84.3) to the overall elimination of bilirubin are unknown, although *in vitro* and *in vivo* studies suggest that photoisomerization is more important than photodegradation [34]. Bilirubin elimination depends on the rates of formation as well as the rates of clearance of the photo products. Photoisomerization occurs rapidly during phototherapy, and isomers appear in the blood long before the level of plasma bilirubin begins to decline. The radiometric quantities used and the important factors that influence the dose and efficacy of phototherapy are listed in Tables 84.5 and 84.6 and illustrated in Fig. 84.4. A common misconception is that ultraviolet (UV) light (< 400 nm) is used for phototherapy. Phototherapy lights in current use do not emit significant erythemal UV radiation. In addition, the glass of the fluorescent tube, the plastic cover of the lamp and, in the case of preterm infants, the incubator, filter out UV light.

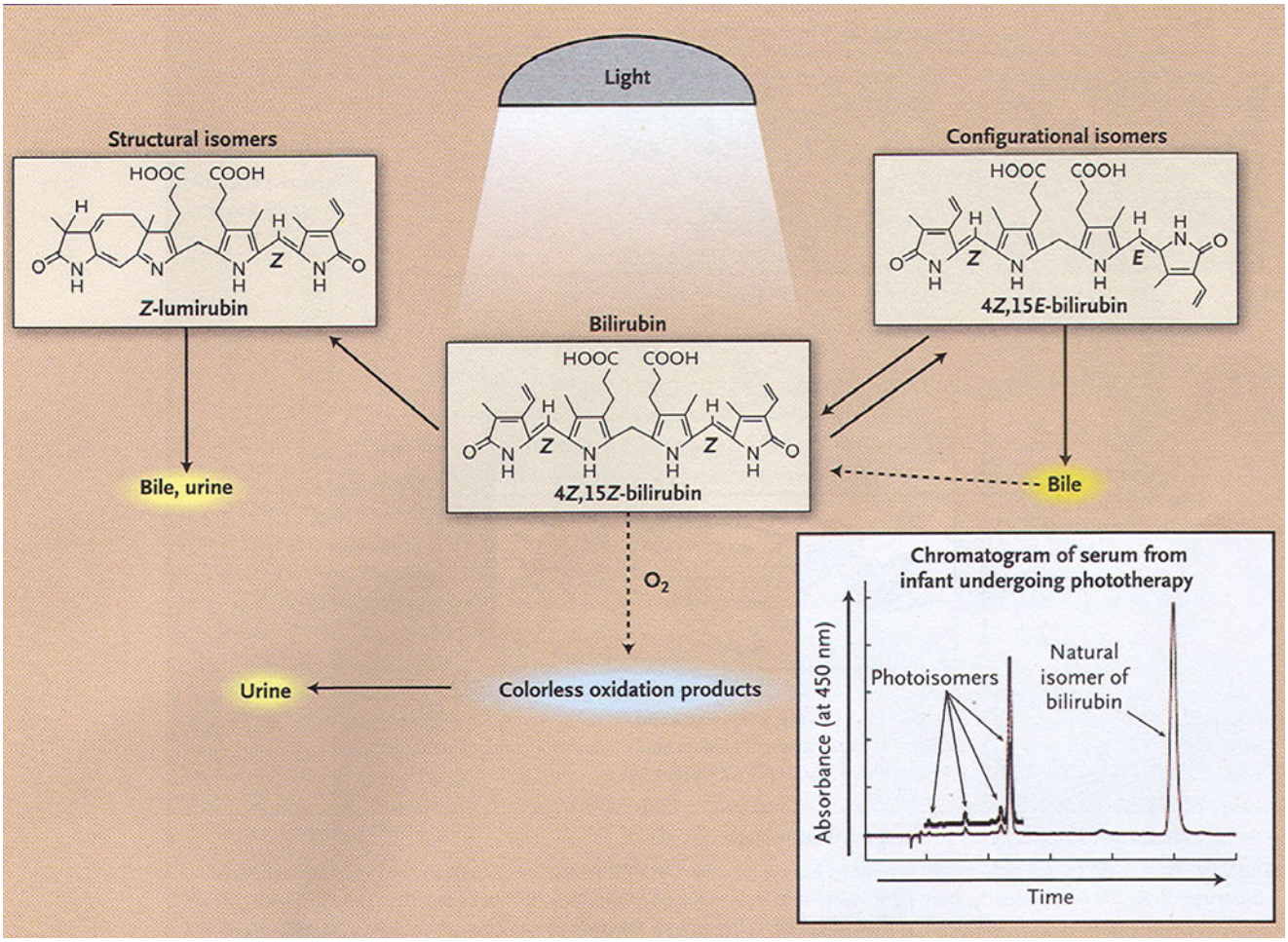

Fig. 84.3 Mechanism of phototherapy. The absorption of light by the normal form of bilirubin (4*Z*,15*Z*-bilirubin) generates fleeting transient excited-state bilirubin molecules. These fleeting intermediates can react with oxygen to produce colorless products of lower molecular weight, or they can undergo rearrangement to become structural isomers (lumirubins) or configurational isomers in which the configuration of at least one of the two *Z*-configuration double bonds has changed to an *E*-configuration. (*Z* and *E*, from the German *zusammen* (together) and *entgegen* (opposite), respectively, are prefixes used for designating the stereochemistry around a double bond. The prefixes 4 and 15 designate double-bond positions). Only the two principal photoisomers formed in humans are shown. The photoisomers are less lipophilic than the 4*Z*,15*Z* form of bilirubin and can be excreted unchanged in bile without undergoing glucuronidation. Lumirubin isomers can also be excreted in urine. Photooxidation products are excreted mainly in urine. Once in bile, configurational isomers revert spontaneously to the 4*Z*,15*Z* form of bilirubin. Configurational isomerization is reversible and much faster than structural isomerization, but the clearance of the 4*Z*,15*E* isomer is slow. Structural isomerization is irreversible and lumirubin is cleared more rapidly and makes the major contribution to overall bilirubin elimination. Both configurational and structural isomerization occur much more quickly than photooxidation. The graph, a high-performance liquid chromatogram of serum from an infant undergoing phototherapy, shows the presence of several photoisomers in addition to the 4*Z*,15*Z* isomer. Photoisomers are also detectable in the blood of healthy adults after sunbathing. From [85], with permission

84.4.1 Clinical Use and Efficacy of Phototherapy

The factors that influence the dose and efficacy of phototherapy are listed in Table 84.6 and illustrated in Fig. 84.4. There is a clear dose-response relationship between the irradiance (energy output of the phototherapy device) delivered to the infant and the rate of decline in the TSB [35] (Fig. 84.5). It is, therefore, important to perform regular measurements of the irradiance with a radiometer or spectroradiometer. Special blue fluorescent lamps or light-emitting diode (LED) lights have been found to be effective in clinical studies [36, 37] and will deliver 30–40 $\mu W/cm^2/nm$ in the 430–490 nm band.

Commonly used phototherapy units include those that contain daylight, white, or blue fluorescent tubes, halogen lamps, and light emitting diodes (LED). When the TSB approaches the range at which exchange transfusion is considered, intensive phototherapy is recommended [22]. The AAP defines intensive phototherapy as the use of high levels of

Table 84.5 Radiometric quantities used in phototherapy

Quantity	Dimensions	Usual units of measure
Irradiance (radiant power incident on a surface per unit area of the surface)	W/m^2	W/cm^2
Spectral irradiance (irradiance in a certain wavelength band)	W/m^2 per nm (or W/m^2)	$\mu W/cm^2$ per nm

From [74] with permission.

Table 84.6 Factors that affect the dose and efficacy of phototherapy

Factor	Technical terminology	Rationale	Clinical application
Type of light source	Spectrum of light (nm)	Blue-green spectrum is most effective at lowering total serum bilirubin (TSB); light at this wavelength penetrates skin well and is absorbed strongly by bilirubin	Use special blue fluorescent tubes or light-emitting diodes (LED) or another light source with output in blue-green spectrum for intensive phototherapy (PT)
Distance of light source from patient	Spectral irradiance (a function of both distance and light source) delivered to surface of infant	↑ irradiance leads to ↑ rate of decline in TSB. Standard PT units deliver 8–10 $\mu W/cm^2/nm$; intensive PT delivers ≥30 $\mu W/cm^2/nm$	If special blue fluorescent tubes are used, bring tubes as close as possible to infant to increase irradiance. (Do NOT do this with halogen lamps because of danger of burn.) Positioning special blue tubes 10–15 cm above infant will produce an irradiance of at least 35 $\mu W/cm^2/nm$
Surface area exposed	Spectral power (a function of spectral irradiance and surface area)	↑ surface area exposed leads to ↑ rate of decline in TSB	For intensive PT, expose maximum surface area of infant to PT. Place lights above and below* or around † the infant. For maximum exposure, line sides of bassinet, warmer bed, or incubator with aluminum foil
Cause of jaundice		Phototherapy (PT) is likely to be less effective if jaundice is caused by hemolysis or if cholestasis is present (direct bilirubin is increased)	When hemolysis is present, start PT at a lower TSB level and use intensive PT. Failure of PT suggests hemolysis is cause of the jaundice. When direct bilirubin is elevated, watch for bronze baby syndrome or blistering
TSB level at start of PT		The higher the TSB, the more rapid the decline in TSB with PT	Use intensive PT for higher TSB levels. Anticipate a more rapid decrease in TSB when TSB > 20 mg/dL

* Commercially available sources for light below include special blue fluorescent tubes available in the Olypmpic Bili-Bassinet (Natus Medical, San Carlos, CA) the BiliSoft Fiberoptic /LED mattress (GE Healthcare, Wauwatosa, WI), NeoBlue cozy mattress (Natus Medical Inc., San Carlos, CA).

† The Mediprema Cradle 360 (Mediprema, Tours Cedex, France) provides 360º exposure to special blue fluorescent light.

irradiance in the 430 to 490 nm band (usually 30 $\mu W/cm^2$ per nm or higher) delivered to as much of the infant's surface area as possible [22]. Thus, it is particularly important to use lamps that have an emission within the 430–490 nm band. Increasing the surface area of the infant exposed to light significantly increases the efficacy of phototherapy and can be achieved by placing additional sources of light below or around the infant (see Table 84.6 for details of some commercially available equipment) [38–40]. A simple method of increasing the area exposed is to place some reflecting material such as a white sheet or aluminum foil within or around the bassinette or incubator [40].

Because phototherapy acts on bilirubin that is present in the extra vascular space as well as in the superficial capillaries, it has become a common practice to turn the infant at intervals from supine to prone and back. Two randomized studies show that turning infants every few hours does not improve the efficacy of phototherapy [41, 42].

84.4.2 Phototherapy to Prevent an Exchange Transfusion

As the primary purpose of phototherapy is to prevent the need for an exchange transfusion, it is useful to know how many infants need to be treated with phototherapy in order to prevent one exchange transfusion.

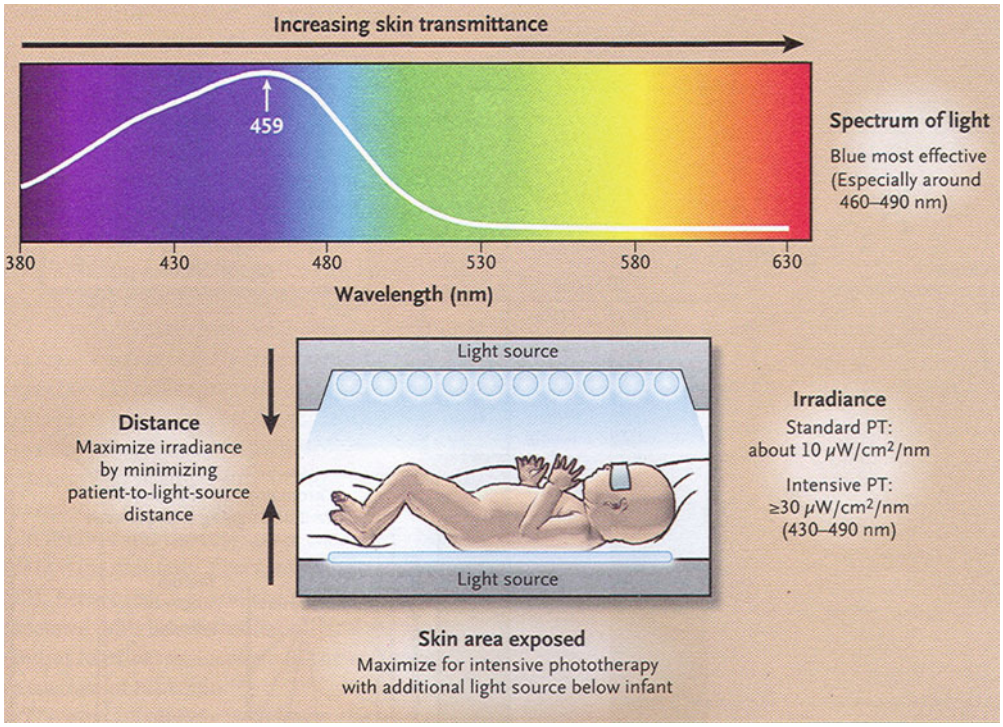

Fig. 84.4 Important factors in the efficacy of phototherapy. The absorbance spectrum of bilirubin bound to human serum albumin (white line) is shown superimposed on the spectrum of visible light. Clearly, blue light is most effective for phototherapy, but because the transmittance of skin increases with increasing wavelength, the best wavelengths to use are probably in the range 460–490 nm. Term and near-term infants should be treated in a bassinet, not an incubator, to allow the light source to be brought to within 10–15 cm of the infant (*except when halogen or tungsten lights are used*), increasing irradiance and efficacy. For intensive phototherapy, an auxiliary light source (fiber-optic pad, light-emitting diode [LED] mattress or pad, or special blue fluorescent tubes) can be placed below the infant or bassinet. Special blue fluorescent tubes can also be placed in a 360° configuration around the infant (see Table 84.6 for a list of some commercially available units). From [85], with permission

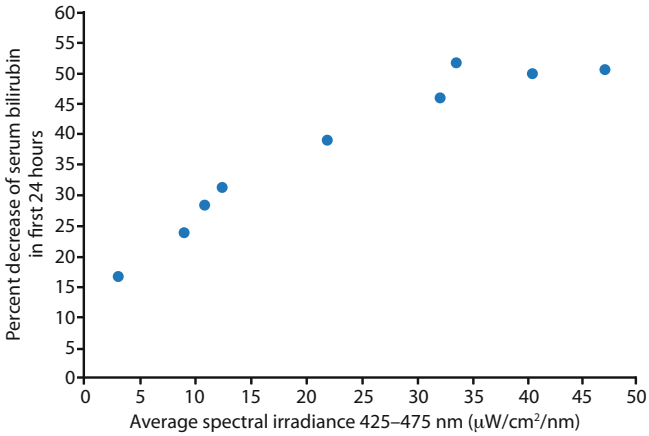

Fig. 84.5 Relationship between average spectral irradiance and decrease in serum bilirubin concentration. Term infants with nonhemolytic hyperbilirubinemia were exposed to special blue lights (Phillips TL 52/20W) of different intensities. Spectral irradiance was measured as the average of readings at the head, trunk, and knees. Drawn from the data of [35]. From [74], with permission

Previous randomized trials of phototherapy [43–45] found that, among infants without hemolysis, 6–10 infants needed to be treated with phototherapy to prevent one from developing a TSB level ≥20 mg/dL (343 μmol/L) [1]. These data, however, are not applicable to the current use of phototherapy because these studies included infants with lower TSB levels than those currently recommended for instituting phototherapy [22] and had as their end points lower TSB levels than those at which the AAP recommends exchange transfusions [22]. A recent analysis of infants ≥ 35 weeks of gestation evaluated the numbers needed to treat (NNT) with phototherapy in order to prevent one infant from reaching the exchange transfusion level according to the current AAP guidelines [46]. The NNT ranged from 10 (95% CI: 6–19) for < 24 hour old, 36 week gestation boys to 3041 (95% CI: 888–11,0096) for ≥ 3 day old 41 week girls [47]. If we need to treat 3000 infants with phototherapy to prevent one exchange transfusion, it is reasonable to ask whether, in many of these infants, phototherapy might have been avoided. In these infants, other interventions including lactation support, formula substitution or supplementation [45], or the use of home phototherapy should also be considered.

84.4.3 Adverse Effects

Reports of clinically significant toxicity from phototherapy are rare and there is no evidence, to date that the products of photodecomposition have any neurotoxic effects [48, 49].

Bronze Baby Syndrome In infants with cholestasis (direct hyperbilirubinemia), phototherapy can produce the bronze baby syndrome, in which the skin, serum, and urine develop a dark grayish-brown discoloration [50, 51]. The pathogenesis of this condition, which occurs only in infants with cholestasis, is not fully understood. When phototherapy is stopped and cholestasis resolves, the coloration disappears.

Skin Rare purpuric and bullous eruptions have been reported in infants with severe cholestatic jaundice who are receiving phototherapy [52, 53], probably as a result of sensitization by accumulating porphyrins. An erythematous rash has been reported in infants treated with tin-mesoporphyrin, who are subsequently exposed to sunlight or daylight fluorescent bulbs [54]. Congenital porphyria, a family history of porphyria, and concomitant use of photosensitizing drugs are absolute contraindications to phototherapy. Severe blistering and agitation during phototherapy could be a sign of congenital porphyria [55]. A recent study suggested that intensive phototherapy might increase the number of atypical melanocytic nevi identified at school age [56] although other research has not shown this association [57].

Insensible water loss and temperature control Conventional phototherapy can produce an acute change in the infant's thermal environment, leading to an increase in peripheral blood flow and insensible water loss [58, 59]. This question has not been studied with LED lights but, because of their relatively low heat output, they should be much less likely to cause insensible water loss. In term infants who are nursing or feeding adequately, additional intravenous fluids are usually not required.

Eye damage Because light can be toxic to the retina, the eyes of infants receiving phototherapy should be protected with appropriate eye patches [60].

Other effects Intensive phototherapy does not cause hemolysis [61]. Because bilirubin is a powerful antioxidant [62, 63], lowering the TSB, particularly in ELBW infants could have undesirable consequences [33] but none have yet been clearly identified although, as noted above, aggressive phototherapy was associated with a non-significant increase in mortality in infants with birth weight 501–750 g [7].

84.4.4 Expected Rate of Decline in Serum Bilirubin

The effectiveness of phototherapy depends not only on the dose of light but also on the cause and severity of the hyperbilirubinemia. During active hemolysis, the TSB may not decline or not decline as rapidly as it would in an infant without hemolysis. On the other hand, because phototherapy works on bilirubin present in the skin and superficial subcutaneous tissue, the more bilirubin present at those sites (i.e., the higher the TSB level) the more effective phototherapy will be [64]. In some infants with a TSB > 30 mg/dL (513 μmol/L), intensive phototherapy can result in a decline of as much as 10 mg/dL (171 μmol/L) within a few hours [65].

Hemolysis is more likely to be the cause of hyperbilirubinemia in infants who require phototherapy during the birth hospitalization than in those readmitted for such treatment [66–68] and phototherapy in infants treated during the birth hospitalization is almost always initiated at a lower serum bilirubin level. For both of these reasons, the level of TSB tends to fall relatively slowly in such infants.

84.4.5 Discontinuing Phototherapy

Although there are no firm standards for discontinuing treatment, phototherapy can be safely stopped in infants treated during the birth hospitalization if two consecutive TSB values fall below the level at which phototherapy was initiated. In infants readmitted for phototherapy, hemolysis is less often the cause for their hyperbilirubinemia [67, 68] and treatment is begun at higher initial TSB levels. In these patients, intensive phototherapy can result in a TSB decrement of 30-40% in the first 24 hours [67] with the most pronounced decline occurring in the first 4–6 hours. Phototherapy can be discontinued when the total serum bilirubin level has fallen below 13–14 mg/dL (222–239 μmol/L) [22].

84.4.6 Rebound After Phototherapy

A rebound in the TSB level of 1–2 mg/dL (17–34 μmol/L) [67], and occasionally more [68], can occur after phototherapy is discontinued. Infants at increased risk of a clinically significant rebound are those born at < 37 weeks' gestation, those with hemolytic disease, and those treated with phototherapy during the birth hospitalization [67, 68]. It is usually unnecessary to keep an infant in the hospital to check for rebound [67, 69] but for infants who require phototherapy during their birth hospitalization and for those with well-defined hemolytic disease, a follow-up bilirubin level should be obtained 24 hours after discharge.

84.4.7 Sunlight Exposure

Sunlight will lower the serum bilirubin level [70] but the practical difficulties involved in safely exposing a naked newborn to the sun either inside or outside (and avoiding sunburn) preclude the use of sunlight as a reliable therapeutic tool.

84.4.8 Home Phototherapy

The economic and social pressures for early discharge of infants from hospital after delivery have led to the widespread use of home phototherapy in the USA. When used appropriately and with adequate monitoring of the TSB, home phototherapy poses no obvious hazards to the infant and is certainly much cheaper than hospital treatment [71, 72]. The development of new LED mattresses and blankets has made it easier and more effective to administer phototherapy at home.

84.5 Exchange Transfusion

The prevention of Rh hemolytic disease with Rh immune globulin and the effective use of phototherapy has led to a dramatic decline in the number of exchange transfusions performed [74]. Of 1974 ELBW infants treated with phototherapy in the NICHD Neonatal Research Network study [7] only 5 required an exchange transfusion (0.25%). It is now possible for a pediatric resident to complete a 3-year training program without ever having performed or even witnessed an exchange transfusion. The indications for exchange transfusions in term and in preterm infants are provided in Tables 84.1–84.3 and in Fig. 84.2.

84.5.1 Exchange Transfusion Risks

The potential complications of exchange transfusions are listed in Table 84.7 and were recently reviewed [1]. The morbidity and mortality associated with exchange transfusions are significantly dependent on the clinical state of the infant before the exchange, being much higher in sick preterm infants and quite low in term and near-term infants who are relatively well [75]. It is interesting that when exchange transfusions were performed using both umbilical venous and arterial catheters, they were significantly more likely to be associated with adverse events than when performed through the umbilical vein alone or via other routes [75].

Table 84.7 Potential complications of exchange transfusion

Cardiovascular	Arrhythmias Cardiac arrest Volume overload Embolization with air or clots Thrombosis Vasospasm
Hematologic	Sickling (donor blood) Thrombocytopenia Bleeding (overheparinization of donor blood) Graft versus host disease Mechanical or thermal injury to donor cells
Gastrointestinal	Necrotizing enterocolitis
Biochemical	Hyperkalemia Hypernatremia Hypocalcemia Hypomagnesemia Acidosis Hypoglycemia
Infectious	Bacteremia Virus infection (hepatitis, CMV) Malaria
Miscellaneous	Hypothermia Perforation of umbilical vein Drug loss Apnea

From [73], with permission.

84.6 Pharmacologic Treatment

Pharmacologic agents used in the management of hyperbilirubinemia can accelerate the normal metabolic pathways for bilirubin clearance, inhibit the enterohepatic circulation of bilirubin, and interfere with bilirubin formation by blocking the degradation of heme or inhibiting hemolysis. Currently the only drug in standard clinical use is intravenous immunoglobulin (IVIG). When administered to infants with isoimmune hemolytic disease IVIG will significantly reduce the need for exchange transfusion [76–78]. The usual dose is 500 mg/kg given over 2–3 hours and, if necessary, repeated in 12 hours. IVIG is recommended if the TSB is rising despite intensive phototherapy or the TSB level is within 2–3 mg/dL (34–51 μmol/L) of the exchange level [22] (Fig. 84.2).

84.6.1 Decreasing Bilirubin Production

The enzyme microsomal heme oxygenase is necessary for the conversion of heme to biliverden, one of the first steps in the formation of bilirubin from heme. Tin-mesoporphyrin (SnMP) is a potent inhibitor of heme oxygenase and is effective in reducing TSB levels and the requirements for phototherapy in term and preterm infants [79]. SnMP has also produced a temporary reduction in TSB levels [80] in patients with the Crigler-Najjar syndrome and has prevented the need for exchange transfusion in Jehovah's Witness' newborns with Rh hemolytic disease [81]. To date, more than 1000 newborns have received SnMP in controlled clinical trials but the drug is still awaiting FDA approval in the USA and currently is the subject of a randomized controlled trial.

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85

Pathology and Treatment of Liver Diseases

Giuseppe Maggiore and Silvia Riva

Liver disease in the newborn usually presents with two principal and distinct syndromes: neonatal cholestasis and neonatal liver failure.

85.1 Neonatal Cholestasis

Cholestasis is related to a reduction of biliary flow, with accumulation in the liver and extrahepatic tissues of substances normally excreted in the bile, such as bilirubin, cholesterol and bile acids. In the newborn, bile acids are physiologically elevated in the serum due to a delayed maturation of biliary secretion. Cholestasis is a pathological condition of a prolonged or permanent disorder of biliary secretion, which is secondary to a wide range of possible causes, such as infections, inborn errors of metabolism, or genetic disorders of biliary transport.

In the neonate, cholestasis usually presents with prolonged jaundice or, less frequently, with a hemorrhagic syndrome related to late onset vitamin K deficiency. Cholestatic jaundice is associated with discolored stools and dark urines due to hyperbilirubinuria. More rarely, cholestasis can present with hypocalcemic seizures due to vitamin D malabsorption and deficiency. Hepatomegaly is a constant feature, while spleen enlargement is observed in about 50% of cases. Pruritus is absent in the neonatal period, but can occur from age 4 months onwards. Every newborn with jaundice that prolongs for more than 2 weeks should be considered a pathological case, and one that needs a precise diagnosis.

Cholestasis affects approximately 1 in every 2,500 infants. Its etiological spectrum is particularly wide, also including conditions with severe prognosis, such as biliary atresia, which is the single most frequent pathologic entity (Table 85.1). The diagnostic approach to prolonged jaundice is as follows:

- Determine its cholestatic nature by looking for an abnormal stool or urine color, and by measuring total and direct serum bilirubin;
- Prevent hemorrhagic complications related to vitamin K deficiency by administering a single 10 mg intramuscular dose of vitamin K1;
- Suspect biliary atresia early if the clinical picture is suggestive since a good outcome of surgery depends on early diagnosis;
- Early referal of the infant to a center experienced in the treatment of hepatobiliary disorders in childhood.

(See also Chapter 86)

85.2 Principal Causes of Cholestasis in the Newborn

85.2.1 Biliary Atresia

Biliary atresia (BA) is an obliterative cholangiopathy of unknown origin involving intra- and extrahepatic biliary structures. Its prevalence in Europe and the USA varies from 1:14,000 to 1:20,000 live born. BA spontaneously progresses to cirrhosis and liver failure in the first years of life. Its treatment is principally surgical and is based on two sequential procedures: the Kasai procedure or hepato-porto-enterostomy corresponding to a bilio-digestive anastomosis between the porta hepatis and an intestinal Roux loop. If this procedure fails, liver transplantation is perfomed [1].

BA seems to be a phenotype caused by several etiologies, including a perinatal insult that initiates an immune-mediated obliteration of the extra hepatic bile duct lumen and an embryonic or fetal defect in the normal morphogenesis of the biliary tree.

The success of the hepatoportoenterostomy depends mainly on the precocity of the diagnosis and surgery [2]. Unfortunately in the early phase of the disease, the newborn

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 85.1 Neonatal cholestasis: the spectrum of etiology

Site of the lesion	Disease
Whole biliary tree	– Biliary atresia – Sclerosing cholangitis with neonatal onset – Sclerosing cholangitis and icthyosis due to claudin I defect
Intrahepatic biliary tree	• Infections – Prenatal: CMV, toxoplasmosis, syphilis, rubeola – Perinatal: disseminated bacterial infections – Postnatal: *E. coli* pyelonephritis • Genetic cholestasis – Progressive familial intrahepatic cholestasis (PFIC) – Benign recurrent intrahepatic cholestasis (BRIC) – alfa-1-antitrypsin deficiency – Cistic fibrosis – Familial cholestasis of native Americans (Quebec) – Niemann-Pick tipo II disease – Gaucher disease – Mucopolisaccaridosis type VI and VII – Mucolipidosis II – Peroxisomal disorders (Refsum disease, Zellweger disease) – Hepatocyte nuclear factor 1β HNF 1β) deficiency – Mithocondrial respiratory chain disorders – Citrin deficiency – Complex III assembly defect (GRACILE syndrome) – Inborn error of primary bile acids synthesis • Syndromic cholestasis – Alagille syndrome – Aagenas syndrome – Williams syndrome – Kabuchi syndrome – Hardikar syndrome – McCune-Albright syndrome – ARC (arthrogryposis, renal dysfunction, and cholestasis) syndrome – Donohue syndrome (Leprecaunism) – McCune-Albright syndrome • Benign intrahepatic colestasis • Other forms of intrahepatic cholestasis – Parenteral nutrition induced-cholestasis – Cholestasis secondary to congenital hypopituitarism – Cholestasis secondary to maternal hypothyroidism – Cholestasis secondary to hepatic hemangiomatosis – Cholestasis secondary to placentar chorioangioma – Cholestasis associated with congenital microvillous atrophy – Cholestasis secondary to fetal post-hemolytic (fetal erytroblastosis) cholestasis – Cryiptogenic intrahepatic cholestasis – Neonatal lupus
Extrahepatic biliary tree	– Choledocholithiasis – Spontaneous perforation of bile ducts – Congenital choledocal strenosis – Congenital choledocal dilatation (coledochal cyst)

appears healthy, meconium is rarely discolored, stools are only moderately discolored, and jaundice is mild. In regions with a high prevalence of BA, such as Taiwan (1:2,700), a strategy based on a universal screening using a stool color card has been successfully developed [3]. The outcome of the Kasai procedure depends on early surgery before the age of 6 weeks, and on the caseload of the center performing the primary surgery. Long-term results of the Kasai procedure show a 10-year survival rate in about 30% of patients, and a 20-year survival rate in about 25%. At present, sequential treatment for biliary atresia results in long-term survival, with a good quality of life in more than 90% of treated patients.

85.2.2 Genetic Cholestasis

Genetic cholestasis includes a heterogeneous group of rare disorders of hepatobiliary transport. This is sometimes restricted to specific geographic areas, such as the case of Aagenas syndrome (also known as Norwegian cholestasis), in which recurrent cholestasis with neonatal onset is associated with lymphedema, or native North American Indian childhood cirrhosis, a familial intrahepatic cholestasis described in Ojibway-Cree children from Quebec, typically presenting with transient neonatal jaundice in an otherwise healthy infant, but progressing to biliary cirrhosis. This disorder is associated with mutation of the FLJ14728 gene (also known as Cirhin), a protein preferentially expressed in the embryonic liver, and of still unknown function.

Bile salts take part in an efficient enterohepatic circulation in which most of the secreted bile salts are absorbed in the terminal ileum. Canalicular secretion is the driving force behind the enterohepatic cycling of bile salts, and most genetic diseases are caused by defects of canalicular secretion.

Progressive familial intrahepatic cholestasis (PFIC), once known as Byler disease, results from mutations in various genes that encode hepatobiliary transport proteins [4]. Mutations in the FIC1 gene (also known as ATP8B1) cause relapsing or permanent cholestasis. The relapsing type of cholestasis is called benign recurrent intrahepatic cholestasis (BRIC type 1), the permanent type of cholestasis is PFIC type 1. PFIC type 2 results from mutations in the bile salt export pump (BSEP) gene (also known as ABCB11). This condition is associated with permanent cholestasis from birth. Serum gamma-glutamyltransferase (γGT) activity is low to normal in PFIC and in BRIC types 1 and 2. PFIC type 3 is caused by mutations in the MDR3 gene (also known as ABCB4) coding for a phospholipid translocator in the canalicular membrane. Because of their inability to secrete phospholipids, patients with PFIC type 3 produce bile acid-rich toxic bile that damages the intrahepatic bile ducts. Serum γGT activity is elevated in these patients. Ursodeoxycholic acid therapy is useful for patients with a partial defect. Liver transplantation is a more definitive therapy for these patients.

Inborn errors of bile acid synthesis are rare genetic disorders that can present as neonatal cholestasis. These disorders are characterized by a failure to produce normal bile acids, with accumulation of unusual bile acids and/or bile acid intermediaries. Individuals with inborn errors of bile acid synthesis generally present with cholestasis and low or normal serum bile acid concentrations, normal γGT serum activity, and the absence of pruritus. Failure to diagnose any of these conditions can result in liver failure or progressive chronic liver disease. If recognized early, many patients have an excellent clinical response to oral bile acid therapy.

Cholesterol is converted to cholic acid and chenodeoxycholic acid through a series of reactions involving modifications of the steroid nucleus and oxidation of the side chain. Inborn errors have been shown for single enzymes involved in modification of the sterol nucleus, and in steps in modification of the side-chain. Unusual bile acids or bile alcohols can be identified using gas chromatography-mass spectrometry and fast atom bombardment mass spectrometry techniques. Two defects that influence the modifications of the steroid nucleus have been well characterized: the 3 beta-hydroxy-delta 5-C27-steroid dehydrogenase deficiency (the most frequent), and the 3-oxo-delta 4-steroid 5 beta-reductase deficiency. Diagnosis of the latter can be problematic because a similar pattern of metabolite excretion can occur as a result of liver damage caused by viruses or inborn errors of pathways unrelated to bile acid synthesis. The 3 beta-hydroxy-delta 5-C 27-steroid dehydrogenase catalyzes an early step in the synthesis of bile acids from cholesterol. Patients with autosomal recessive mutations in the encoding gene HSD3B7 fail to synthesize bile acids, and develop cholestatic jaundice and malabsorption of lipids [5]. Pathological findings may include intralobular cholestasis with giant cell transformation, and hepatic injury confined to the portal limiting plate where the smallest bile ductules may be injured, and where fibrosis typically develops. Interlobular bile ducts are usually spared. Ultrastructure of the liver reveals nonspecific change. Liver disease improves dramatically on treatment with chenodeoxycholic acid, which produces beneficial feedback inhibition of abnormal bile acid production and enhances choluresis.

Defective side chain oxidation in patients with cerebrotendinous xanthomatosis (CTX) leads to synthesis of bile alcohols, but patients with CTX do not have cholestatic liver disease. Their major problems (neurological disease, atherosclerosis and xanthomata) are caused by an accumulation of cholestanol and cholesterol in the tissues, while bile acid precursors are probably diverted into the synthesis of cholestanol. Chenodeoxycholic acid suppresses the production of abnormal metabolites from cholesterol (by inhibition of cholesterol 7 alpha-hydroxylase) and leads to improvement in the neurological condition. Defective side chain oxidation also occurs in peroxisomal disorders, leading to accumulation of such C27 bile acids as trihydroxycoprostanic acid (THCA).

Alagille syndrome (AGS) is an autosomal-dominant disorder with variable penetrance, and a prevalence of approximately 1 in 70,000 live births [6]. AGS may present in the neonatal period with cholestasis that may be severe enough to suggest biliary atresia. AGS is characterized by five principal features: chronic cholestasis resulting from paucity of interlobular bile ducts; peripheral pulmonary artery stenosis, butterfly-like vertebral arch defect, posterior embryotoxon, and a peculiar *facies* (Fig. 85.1). In the complete form of the syndrome, all five features are observed, though incomplete or partial forms exist. Other less frequent features include growth retardation, renal and bone abnormalities, ocular abnormalities – such as optic disc drusen, angulated retinal vessels, pigmentary retinopathy, and vascular intracranic abnormalities.

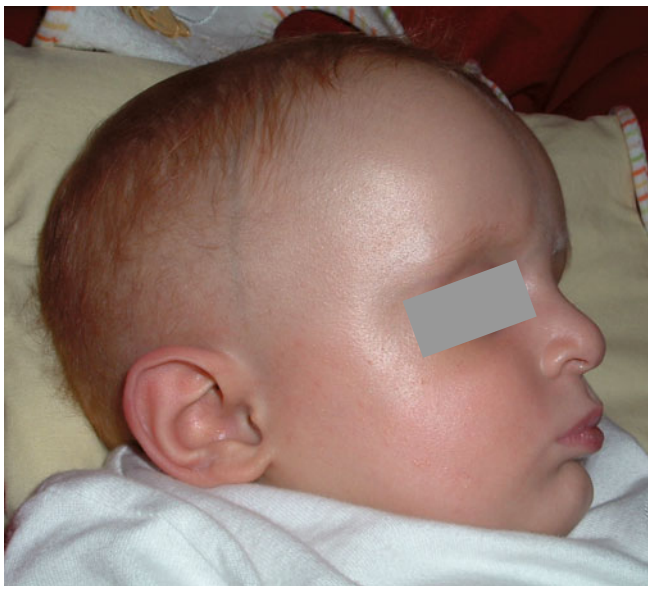

Fig. 85.1 Alagille syndrome: typical facies

Jagged1 (JAG1) has been identified as the gene responsible for coding for a cell surface protein that functions in an embryologically important signaling pathway, known as the Notch signaling pathway. Defects of the Notch pathway can impair angiogenesis. Mutations in JAG1 can be identified in at least 70% of AGS, though they are inherited in only 30% of cases. These JAG1 mutations include total gene deletions, as well as mutations (frameshift, missense, and nonsense) in almost all regions of the gene.

Therapy consists of nutritional supplementation with medium-chain triglycerides, essential fatty acids and fat-soluble vitamins. Liver transplantation has been used successfully to treat patients with liver failure, portal hypertension or severe pruritus and xanthomatosis (Fig. 85.2).

Alpha-1-antitrypsin deficiency (AATD) is a genetic disorder that can manifest as neonatal cholestasis or chronic liver disease in infancy and childhood, pulmonary emphysema in adulthood, and, more rarely, as skin panniculitis or membranoproliferative glomerulonephritis [7]. AATD is caused by mutations in the SERPINA1 gene encoding

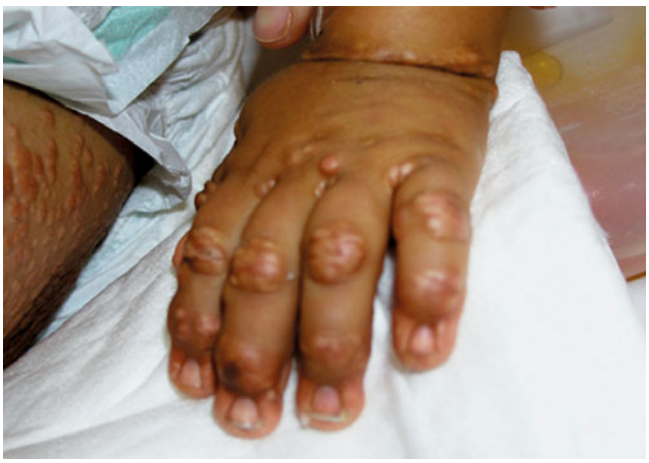

Fig. 85.2 Alagille syndrome: diffuse xantomata

alpha-1-antitrypsin (AAT), and is inherited as an autosomal recessive trait. AATD is characterized by low serum levels of AAT, the main protease inhibitor (PI) in human serum. The prevalence in Western Europe and in the USA is estimated at approximately 1 in 2500/5000 newborns, most common amogst those of Scandinavian descent. The most common deficiency alleles in North Europe are PI Z and PI S, and the majority of individuals with severe AATD are PIZZ. The mutant Z protein product is synthesized in hepatocytes, and then accumulates intracellularly rather than being appropriately secreted, causing hepatocellular injury. Ten percent of PI-ZZ AATD infants present with cholestatic jaundice with an incidence of 1:10,000/20,000 newborns. However, only 5% develop a severe progressive cholestatic liver disease that requires early liver transplant. In the majority of cases, clinical jaundice disappears, but clinical and biochemical features of chronic liver disease may persist and progress to biliary cirrhosis in the pediatric age. The variable clinical presentations suggest an important contribution of genetic and environmental disease modifiers. In PIZZ homozygotes, specific globules, which are due to accumulation of a type of AAT, are seen in liver cells. Ursodeoxycholic acid therapy may significantly improve clinical status and liver test results in some children with liver disease associated with AATD. No beneficial effect of UDCA has been shown in children with the most severe liver involvement: these patients are candidates for liver transplantations.

85.3 Management of a Cholestatic Newborn

The main goal of management of an infant with cholestasis is to detect or at least to exclude biliary atresia within 45–60 days of age [8]. History and physical examination, together with simple laboratory tests and a liver ultrasound, will allow clinicians to achieve this in the majority of cases. Decolorized stools and a hard palpable liver strongly suggest BA, even though some infants with BA have pigmented stools at presentation.

There is no single laboratory test to confirm or exclude BA. However, biochemical evaluation will allow clinicians to [8]:

- Confirm the cholestatic nature of the jaundice by measuring total and direct bilirubin. A serum direct bilirubin value of >1.0 mg/dL (if the total bilirubin is <5 mg/dL), or a direct bilirubin of >20% of the total bilirubin (if the total bilirubin is >5 mg/dL), suggests cholestasis that can be confirmed by measuring the total serum bile acids levels. A non-cholestatic increase of direct bilirubin is present in Dubin-Johnson syndrome, an autosomal recessive inherited inborn error of bilirubin transport due to mutation of the MRP2 gene;
- Distinguish a cholestatic jaundice from a jaundice related to liver failure where a non-vitamin-K-dependent coagulopathy exists;
- Exclude other causes of cholestasis, such as alpha-1-antitrypsin deficiency, by examining the protein electrophoretic pattern or cystic fibrosis by a normal sweat test. A normal eye examination and a column x –ray showing absence of butterfly vertebrae are against a diagnosis of Alagille syndrome.

In BA, total bilirubin serum levels do not generally exceed 6–8 mg/dL, aminotransferase activity is generally increased, with a non-specific pattern, and alkaline phosphatase activity measurement is not helpful because of the high activity physiologically present in the newborn infant.

Gamma glutamyl transpeptidase (γGT) has been used in the past to distinguish biliary atresia from intrahepatic cholestasis, but wide variability in serum activity makes interpretation of test results difficult. The degree of elevation of γGT is not useful in discriminating the etiology of the cholestasis, even if a normal γGT level suggests genetic and metabolic causes of intracellular cholestasis [9].

Serum bile acids are, by definition, elevated in cholestasis. Light to moderate increase in cholestatic infants with low to normal γGT level suggests an inborn error of bile acids synthesis. Urinanalysis and culture are diagnostic in case of cholestasis secondary to an *E. coli* urinary infection.

Ultrasonography is recommended for the diagnostic evaluation of the infant with cholestasis. Ultrasound is operator dependent, and thus should be performed at a referral center by experienced personnel. Ultrasound is particularly useful in identifying anatomic abnormalities, such as abnormal dilatation of extrahepatic bile ducts. The finding of a small or absent gallbladder may suggest BA, but cannot be used to rule out this diagnosis. The presence of a visible gallbladder does not allow the exclusion of BA. The presence of features commonly associated with BA, such as poly/asplenia, preduodenal portal vein, complete situs inversus or cystic dilatation at porta hepatis, may suggest the diagnosis. Several reports on the high sensitivity and specificity of the triangular cord sign on ultrasound suggest that this test may be useful in the diagnosis of BA.

Hepatobiliary scintigraphy is time consuming, expensive, and generally adds little to the routine evaluation of the cholestatic infant because of its low specificity (a significant number of false-positive and false-negative results of the test).

Percutaneous liver biopsy can be performed safely in young infants and can provide disease-specific findings. Examples include PAS-positive granules in alpha-1-antitrypsin deficiency, ductal paucity in Alagille syndrome, necroinflammatory duct lesions in sclerosing cholangitis, and other findings that are relatively specific for metabolic and storage diseases. Liver biopsy has a very high sensitivity and specificity for the diagnosis of BA. However, because of the dynamic and progressive nature of extrahepatic biliary atresia, even this test can be misleading. Biopsy interpretation is pathologist-dependent, and requires expertise in pediatric liver disease [10].

85.4 Treatment of a Cholestatic Newborn Infant

Specific medical treatment of neonatal cholestasis is directed at the cause and may be associated with urinary tract infection, hypothyroidism, hypopitiutarism congenital toxoplasmosis or syphilis.

Ursodeoxycholic acid, particularly at a high dose (30 mg/kg/day) has been proven to be effective and safe in some cholestatic conditions of infancy, such as parenteral nutrition-associated cholestasis, progressive intrahepatic cholestasis of infancy (particularly of type 3), alpha-1-antitripsin deficiency, cystic fibrosis, biliary atresia after successful Kasai procedure, and post-hemolytic cholestasis.

If there is no specific therapy, treatment is supportive and consists primarily of nutritional therapy, including supplements of vitamins A, D, E, and K.

In infants with chronic cholestasis, replacement therapy of the fat-soluble vitamins may prove extremely difficult because of a low concentrations of intraluminal bile acids. Water-soluble forms of vitamin E, d-alpha-tocopheryl polyethylene glycol-1000 succinate, have enabled correction of vitamin E deficiency states in these patients. Exclusively breastfed infants are at particular risk of hypovitaminosis D, and at even greater risk in the absence of adequate exposure to sunlight or when the maternal diet is not sufficient to provide for vitamin D requirements.

Vitamin K prophylaxis at birth by oral or intramuscular administration of 1 mg of vitamin K effectively prevents vitamin K deficiency bleeding (VKDB), formerly known as hemorrhagic disease of the newborn. In exclusively breast-fed infants, a single intramuscular dose at birth effectively prevents late VKDB. Infants with cholestasis are not fully protected in this way and should be given supplements.

For formula-fed infants, a formula that is high in medium-chain triglycerides should be used because it is better absorbed in the presence of bile salt deficiency. Adequate calories and protein are required: infants may need more than 130 calories/kg day and 2–3 g of protein/day.

Infants with persistent cholestasis are at a high risk for essential fatty acid (EFA) deficiency, particularly of the omega-6 fatty acids linoleic and arachidonic acid. EFA deficiency appears to be related to fat malabsorption, hepatic EFA metabolism, enhanced lipid peroxidation and dietary intake. In case of severe itching, rifampin (10 mg/kg/day) can be administered.

85.5 Neonatal Acute Liver Failure

Neonatal acute liver failure (NALF) is a rare and challenging condition. NALF is a multietiologic syndrome that is difficult to recognize initially. As important as the recognition of causes that may indicate specific diet and medical therapy, is the selection of infants suitable for liver transplantation, given that mortality is high and only 25% of infants survive with their own liver. Management of NALF requires supporting the neonate until liver regeneration or liver transplantation takes place.

85.5.1 Definition

The definition of NALF is controversial. In adults, acute liver failure is defined by the onset of hepatic encephalopathy within 8 weeks of the first symptoms of illness, generally jaundice, in the absence of pre-existing liver disease. This definition is unsatisfactory in infancy because encephalopathy is a late event in the course of the disease. Furthermore, the duration of illness can be difficult to assess, particularly in infants who present with ALF in the first few weeks of life secondary to a condition that may be caused by unrecognized metabolic diseases. For these reasons, the Pediatric Acute Liver Failure Study Group has came to a consensus agreement, defining ALF in infancy as a coagulopathy due to liver dysfunction not corrected by vitamin K administration in a patient without a history of known chronic liver disease, and with an INR greater than 1.5 if the patient has encephalopathy, or greater than 2.0 if the patient does not have encephalopathy [11]. In the neonate, ALF can be defined by the presence of severe coagulopathy with a prothrombin time of >17 seconds and/or a factor V of < 50%, with an onset in the first 4 weeks of life, regardless of the presence of a clinical hepatic encephalopathy [12,13].

85.5.2 Etiology

NALF can be classified according to the timing of the onset of liver damage. if the onset is during fetal life, chronic liver damage develops with decompensation at birth. If there is

Table 85.2 Causes of neonatal acute liver failure

Fetal onset
- Neonatal hemochromatosis
- Hemophagocytic lymphohistiocytosis
- Mithocondrial respiratory chain disorders
  - Mithocondrial hepatopathy
  - Mithocondrial DNA depletion syndrome
  - Complex III/IV (cytochrome oxidase) assembly defect
- Inborn disorders of bile acids synthesis
  - Oxisterol 7α-hydroxylase deficiency
  - Di/trihydroxy-cholestanoic acidemia
  - Delta 4-3-Oxosteroid 5-β–reductase deficiency
- Neonatal lupus
- Neonatal veno-occlusive disease
- Neonatal leukemia

Perinatal onset
- Viral infections
  - Enterovirus
    - Echovirus 11
    - Echovirus 6 and 9, Coxackie
  - Herpes virus
    - Herpes simplex virus 1 and 2
    - HHV6
  - Parvovirus B19, Adenovirus, Paramixovirus
- Inborn metabolic disorders
  - Galattosaemia
  - Hereditary tyrosinaemia type 1
  - Hereditary fructose intolerance
  - Urea cycle disorders
- Bacterial infections
- Drug
- Ischemic (shock liver syndrome)
- Undeterminated

damage in the perinatal period, acute liver damage develops at birth without underlying liver disease. Table 85.2 lists most of the recognized causes of NALF. The most common etiologies are liver-based metabolic disorders and perinatally acquired infections [14].

85.5.2.1 Neonatal Hemochromatosis

Neonatal hemochromatosis (NH) is a rare and severe disorder associated with massive intrahepatic and extrahepatic (pancreas, heart, thyroid, salivary glands) abnormal iron storage (siderosis) that spares the reticuloendothelial system [15]. Liver injury has been identified as early as 28 weeks of gestation. The etiology of this condition is unclear, and probably an endpoint of different (viral, immununological) intrauterine insults of fetal liver. The recurrence within sibships at a rate higher than that predicted for simple Mendelian autosomal-recessive inheritance suggests a role for a maternal factor, and maternal alloimmunity has been hypothesized as a cause. Moreover, immunomodulation during at-risk pregnancy appears to lessen the severity of disease. Previous miscarriages are generally recorded and oligohydramnios or polyhydramnios can complicate the pregnancy. Neonates are often premature and/or small for dates. The usual presentation is of acute decompensation of end-stage liver disease in the first few days of life with hypoglycemia, coagulopathy, hyperammonemia, jaundice, irritability and/or drowsiness and ascitis.

The diagnosis of NH must be considered in any neonate with coagulopathy. Diagnosis is based on the exclusion of other causes of liver disease, such as neonatal lupus and hemophagocytic lymphohistiocytosis, and by evidence of iron overload with high serum ferritin, high trasferrin saturation (>95%), and by MR imaging of the liver and pancreas. When feasible, liver biopsy shows grade 3–4 siderosis in the hepatocyte. Biopsy of minor salivary gland is simpler for evidencing extrahepatic siderosis. The prognosis is poor. There have been some reports of spontaneous recovery, and of recovery with anti-oxidant therapy and iron chelation (Table 85.3) in those with a milder phenotype. Liver transplantation has been performed successfully to treat the disease.

Table 85.3 Etiology and diagnostic screening in case of neonatal liver failure

Etiology	Diagnostic screening
Infections	
Enterovirus	Viral culture and PCR detection in blood, urine, stools, CSF
HSV 1 and 2	
HHV6	Viral serology in mother and infant
Adenovirus	
Parvovirus B19	
Bacterial	Blood culture
Metabolic	
Galactosemia	Erytrhrocyte galactose-1-phosphate concentration. GALT activity
HFI	Mutations of HFI gene
HT1	Plasma tyrosine, phenylalanine, methionine and succinyl acetone in urine
MRCD	
IE bile acids	Total serum bile acids; FAB-MS and GC-MS urine analysis
Urea cycle disorders	Plasma aminoacids; urinary orotic acid
CDGS	Transferrin iso-electrofocusing
NH	Serum ferritin levels, saturation of transferrin; MRI
Infiltrative/storage	Bone marrow examination
HLH	
Leukemia	
Nieman Pick type II	
Others	Maternal autoantibodies of the Ro/La family
Neonatal lupus	
Drug	
Hypoxic/ischemic	

HSV herpes simplex virus, *HHV6* human herpes virus 6, *HFI* hereditary fructose intolerance, *HT1* hereditary tirosinemia type 1, *MRCD* mithocondrial respiratory chain disorders, *IE* bile salts: inborn errors of bile salt synthesis, *CDGS* congenital disorders of glycosylation syndrome, *NH* neonatal hemochromatosis, *HLH* hemophagocytic lymphohistiocytosis, *GALT* galactose-1-phosphate urydyl transferase, *FAB-MS* fast atom bombardement ionisation-mass spectrometry, *GC-MS* gas chromatography-mass spectrometry, *MR* magnetic resonance imaging, *CSF* cerebrospinal fluid.

85.5.2.2 Familial Hemophagocytic Lymphohistiocytosis

Familial hemophagocytic lymphohistiocytosis (FHLH) is a rare disorder, with autosomal recessive transmission involving inappropriate activation of macrophages. Clinical features include fever, marked liver and spleen enlargement and neurological abnormalities. Laboratory investigations show pancytopenia, hypertrigliceridemia, severe coagulopathy with hypofibrinogenemia, abnormal liver enzymes and cerebrospinal fluid pleiocytosis (in 50% of cases). The natural killer activity of the peripheral blood lymphocytes is severely depressed. The diagnosis is difficult because hemophagocytosis can be absent in bone marrow aspirate in the early phases of the disease. Initial management includes chemotherapy with dexamethasone, etoposide (VP 16) and cyclosporin, but the prognosis is poor, with a 5-year survival rate of 21%.

Long-term survival requires a bone marrow transplant with a matched sibling, a treatment with a 5-year survival rate of 68%. Liver transplantation is contra-indicated in this condition due to recurrence in the graft.

85.5.2.3 Mitochondrial Respiratory Chain Disorders

Most infants have an early onset with non-specific symptoms (lethargy, hypotonia, poor feeding) and/or extrahepatic features (neurological symptoms, myopathy, proximal renal tubular dysfunction, and hypertrophic cardiomyopathy, hematological and gastrointestinal disorders). A few patients with respiratory chain defects limited to the liver have been described. Thorough investigation is necessary to exclude significant extrahepatic involvement before OLT can be proposed. Liver dysfunction may precede neurologic involvement for weeks, months, or years. Laboratory tests supporting the diagnosis are increased lactate and ketone bodies, a 3-OH-butyrate: acetoacetate ratio >2, and the presence of 3-metylglutacoic aciduria.

85.5.2.4 Herpes Simplex Virus (HSV 1 and 2)

Neonatal herpes simplex virus (HSV 1 and 2) infection can be transmitted at delivery because of exposure to infected maternal genital secretions or lesions, although intrauterine or postnatal infection can also occur. The risk of transmission is highest in the case of primary infection in the mother, who is asymptomatic in most (60–80%) cases. The incidence is 1:3,500 to 1:20,000 live newborns, with a higher (70%) prevalence of HSV type 2. Delivery by cesarean section significantly reduces the risk of neonatal infection. Symptoms typically develop after day 5. ALF may occur within a disseminated disease involving skin, eyes, mucous membranes, brain, lung, adrenals and liver or as the only manifestation. Despite acyclovir therapy, the prognosis is poor, with a high mortality (50–70%) and morbidity (neurologic sequelae). Successful liver transplantation has been reported.

85.5.2.5 Hepatitis B Virus

Hepatitis B virus is vertically transmitted during delivery from a viremic mother. Infants at risk of developing ALF are those born to an HBeAg negative anti-HBe positive, but HBsAg positive mother. ALF usually develops after the neonatal period and within 12 weeks of life. Universal screening of HBsAg positive mothers and passive active immunizations by three or four doses of HBV vaccine in infancy and hepatitis B immunoglobulin within 24 hours of birth are effective in preventing HBV infection of the newborn babies independently of the HBe antigen status of the mother. In areas with a low prevalence of HBV infection or limited resources, three doses of HBV vaccine universally in infancy, instead of maternal screening, can also produce good protection.

85.5.2.6 Other Viruses

Human herpes virus 6 (HHV6) infection has been reported occasionally. Other viruses such as Adenovirus, Parvovirus B19, Paramyxovirus have also been associated with hepatic failure. Among enteroviruses, Echovirus, particularly serotype 11, is the most frequently identified. Severe infection with multi-organ involvement, including the liver, occurs almost exclusively in newborns, typically between days 4 and 7. Mortality is high, but antiviral treatment with an antiviral agent pleconaril, 15 mg/kg/d may improve the outcome.

85.5.2.7 Inborn Metabolic Disorders

Classic galactosemia is an autosomal recessive disorder because of a deficiency of the enzyme galactose-1-phosphate uridyl transferase. The onset usually occurs early in the neonatal period and complete elimination of dietary lactose/galactose results in the resolution of liver failure.

Hereditary fructose intolerance (HFI) is an autosomal recessive disorder caused by a deficiency of the enzyme fructose-1-phosphate aldolase. Symptoms develop only once fructose and sucrose have been introduced into the diet. Thus symptoms do not usually occur in neonates who are fed with fructose- and sucrose-free milk formulas, except for those given medications or rehydration formulas containing fructose, sucrose or sorbitol. Complete elimination of fructose, sucrose and sorbitol results in a dramatic improvement within 2 days.

Hereditary tyrosinaemia type 1 (HT1) is an autosomal recessive disorder caused by a deficiency of fumarylacetoacetate hydrolase, which results in accumulation of toxic metabolites responsible for liver failure, proximal renal tubular dysfunction, and porphyria-like crises. Infants most often present with a coagulopathy soon after birth. Clinical signs of liver failure become apparent usually between 1 and 6 months of age. Diagnosis is based on the finding of succynil

Table 85.4 Anti-oxidant cocktail used in management of neonatal hemochromatosis

– N-acetylcysteine (140 mg/kg orally, then 70 mg/kg 4 hour for 19 doses)
– Selenium (3 μg/kg/day) intravenously
– Prostaglandin E1 (0.5 μg/kg/h) intravenously
– Desferioxamine 30 mg/kg day intravenously over 8 hours until ferritin < 500 μg/L
– Alpha tocopheryl polyethylene glycol succinate (20–30 IU/kg/day)

acetone in the urine and by enzyme analysis of skin fibroblast. Management includes 2-(2 nitro-4-trifluoromethylbenzoyl)-1,3 cyclohexanedione diet (NTBC therapy) associated with a tyrosine- and phenylalanine-restricted diet, which has been proven curative in 90% of cases. Infants who do not respond to NTBC, or those with a suspicion of hepatocellular carcinoma are considered for liver transplant [16].

85.5.2.8 Inborn Error in Bile Acid Synthesis

The delta 4-3-oxosteroid 5beta-reductase deficiency is the principal defect recognized as a cause of hepatic failure. Early diagnosis is important because oral administration of chenodeoxycholic acid and/or cholic acid can be curative.

85.5.2.9 Ischemic Damage

Transient acute liver dysfunction in the neonate secondary to hemodynamic failure or shock liver syndrome is a well recognized complication of reduced hepatic blood flow in low cardiac output states. The peculiar pattern of liver blood flow in neonates characterized by a large intrahepatic shunt through the ductus venous and low arterial blood supply produces a symmetric response to ischemia, with a significant reduction of blood flow in the right lobe compared to left liver, which is supplied by the umbilical venous blood [17].

85.5.3 Clinical Presentation

The clinical presentation of acute liver failure (ALF) in the neonate depends on the etiology. Presentation at birth implies an intrauterine insult, such as congenital infection, NH or mitochondrial disorders. A later presentation may be related to bacterial or viral infection or a metabolic condition unveiled by the introduction of feeding (e.g. galactosemia, HFI, HT1). A detailed family history, including information on consanguinity, previous miscarriages, neonatal deaths, and liver disease in sibships is important to record. Neonatal ALF may be difficult to recognize initially. Non-specific symptoms such as vomiting, poor feeding, and lethargy are early signs. Jaundice is inconstant, especially when inborn metabolic diseases are involved. Encephalopathy can be a late feature, and is particularly difficult to diagnose in neonates. Irritability and reversal of day/night sleep patterns indicate hepatic encephalopathy. Convulsions may be caused by meningoencephalitic involvement or hypoglycemia. Hepatomegaly is most often present. Splenomegaly and ascites are usually noted in cases of severe diseases progressing to cirrhosis.

85.5.4 Management

Management of neonatal ALF requires intensive support and referral to a pediatric liver unit for early evaluation for liver transplantation. Enteral feeding should be preferred and recommenced as soon as possible, although temporary exclusively parenteral nutrition may be needed.

Galactose- and fructose-free diets should be used until the results of galactose-1-phosphate uridyl transferase and urine succinylacetone are available. Normoglycemia should be maintained by high glucose concentration infused through a central line. Coagulopathy, if severe, must be controlled with the infusion of fresh frozen plasma. Fluids should be restricted to 75% of maintenance to prevent cerebral edema. Ascites may respond to infusion of serum albumin associated with diuretic therapy. Gastrointestinal bleeding should be prevented with ranitidine or omeprazole.

Ventilatory support should be considered early in case of neurological as well as respiratory deterioration. Hepatic encephalopathy should be treated by protein intake restriction to 0.5 g/kg and enteral lactulose.

Wide spectrum antibiotics and fluconazole target on positive cultural findings should be administered even in absence of evidence of sepsis. Intravenous acyclovir should be continued until herpes virus infection has been excluded. Antioxidant cocktail (Table 85.4) may be useful in NH if begun within 48 hours of birth, and in milder cases.

The main causes of neonatal ALF that contraindicate orthotopic liver transplantation are familial hemophagocytic lymphohistiocytosis, neonatal leukemia, mitochondrial disorders with extrahepatic involvement, multi-organ failure and uncontrolled sepsis. Neonatal hemochromatosis and viral ALF are currently the most common indication for liver transplantation in neonatal ALF. Despite the introduction of surgical techniques, such as the split liver, which allow surgeons to use part of a donor liver, the mortality rate remains high for patients on the waiting list for surgery.

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Neonatal Cholestasis-Conjugated Hyperbilirubinemia

Chad M. Best, Glenn R. Gourley and Vinod K. Bhutani

86.1 Introduction

Jaundice during the first few weeks of life is a common finding. Frequently, such jaundice is due to elevation of the unconjugated, or indirect bilirubin, and is often the result of a benign process. More concerning is jaundice due to elevation of the conjugated bilirubin fraction. Neonatal cholestasis is defined as an accumulation of biliary substances, such as bilirubin and bile acids because of impaired canalicular bile flow. Neonatal cholestasis manifests with a conjugated hyperbilirubinemia and must be differentiated from unconjugated hyperbilirubinemia, as it is more often associated with a specific disease process. The incidence of neonatal cholestasis has been estimated at 1 in 2500 live births [1].

86.2 Pathophysiology

The process of bile generation in the liver involves two key processes. The first process involves the uptake of bile acids from the blood into the hepatocyte. Uptake across the sinusoidal membrane of the hepatocyte is an active process and involves two receptors: the Na taurocholate cotransporting polypeptide (NTCP) and the organic anion transporting proteins (OATP). Besides transport of bile acids, these receptors also transport other anions, such as toxins and drugs, across the sinusoidal membrane. The secretion of bile acids across the biliary canalicular membrane involves the bile salt export pump (BSEP) and the multidrug resistant proteins (MRP2 and MDR3).

In neonates, there is structural, as well as functional, immaturity of the biliary system. Such immaturity increases susceptibility to cholestasis. The uptake of bile acids across the sinusoidal membrane can be affected a number of ways, as with down regulation of NTCP and OATP receptors, secondary to sepsis and hepatitis, resulting in cholestasis secondary to decreased bile production. Alteration in the canalicular membrane receptors can similarly result in cholestasis, such as that seen with mutation in the BSEP or MDR3 in the progressive familial intrahepatic cholestasis syndromes.

86.3 Classification of Neonatal Cholestasis

The differential diagnosis of neonatal cholestasis is extensive (Table 86.1). Neonatal cholestasis can be classified according to anatomy or etiology. Anatomically, causes of cholestasis can be classified as extrahepatic or intrahepatic. Examples of extrahepatic causes of cholestasis are choledochal cysts and biliary atresia, whereas idiopathic neonatal hepatitis, congenital infections, and α_1-antitrypsin (α_1-AT) deficiency are examples of intrahepatic causes. Etiologically, the causes of cholestasis can be categorized as infectious, metabolic, toxic, chromosomal, vascular disorders and bile duct anomalies.

86.4 Clinical Presentation

Infants with cholestasis usually present with the classic triad of prolonged jaundice, pale stools, and dark urine. Acholic stools warrant expedient evaluation. The spectrum of clinical presentation can vary from progressively poor feeding, failure to thrive, and hypoglycemia seen with metabolic disease to a picture of acute illness with lethargy, irritability, and poor feeding, in patients with such causes as bacterial sepsis, galactosemia and other metabolic syndromes. Bleeding may be present due to vitamin K deficiency secondary to malabsorption of fat soluble vitamins or from deficiency of clotting factors.

On physical examination, cholestatic infants will exhibit jaundice. Hepatomegaly is common, with splenomegaly a sign of advanced liver disease. A mass may be appreciated in

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 86.1 Differential diagnosis of neonatal cholestasis

1. *Idiopathic neonatal hepatitis*
2. *Infections*
   - Viral
     - Cytomegalovirus
     - Rubella
     - Reovirus3
     - Adenovirus
     - Coxsackie virus
     - Human herpes virus 6
     - Varicella zoster
     - Herpes simplex
     - Parvovirus
     - Hepatitis B and C
     - Human immunodeficiency virus
   - Bacterial
     - Sepsis
     - Urinary tract infection
     - Syphilis
     - Listeriosis
     - Tuberculosis
   - Parasitic
     - Toxoplasmosis
     - Malaria
3. *Bile duct anomalies*
   - Biliary atresia
   - Choledochal cyst
   - Alagille syndrome
   - Non syndromic bile duct paucity
   - Inspissated bile syndrome
   - Caroli syndrome
   - Choledocholithiasis
   - Neonatal sclerosing cholangitis
   - Spontaneous common bile duct perforation
4. *Metabolic disorders*
   - α_1-antitrypsin deficiency
   - Galactosemia
   - Glycogen storage disorder type IV
   - Cystic fibrosis
   - Hemochromatosis
   - Tyrosinemia
   - Arginase deficiency
   - Zellweger's syndrome
   - Dubin-Johnson syndrome
   - Rotor syndrome
   - Hereditary fructosemia
   - Niemann Pick disease, type C
   - Gaucher's disease
   - Bile acid synthetic disorders
   - Progressive familial intrahepatic cholestasis
   - North American Indian familial cholestasis
   - Aagenaes syndrome
   - X-linked adreno-leukodystrophy
5. *Endocrinopathies*
   - Hypothyroidism
   - Hypopituitarism
   - (Septo-optic dysplasia)
6. *Chromosomal disorders*
   - Turner's syndrome
   - Trisomy 18
   - Trisomy 21
   - Trisomy 13
   - Cat-eye syndrome
   - Donahue's syndrome (Leprechauns)
7. *Toxic*
   - Parenteral nutrition
   - Fetal alcohol syndrome
   - Drugs
8. *Vascular*
   - Budd-Chiari syndrome
   - Neonatal asphyxia
   - Congestive heart failure
9. *Neoplastic*
   - Neonatal leukemia
   - Histiocytosis X
   - Neuroblastoma
   - Hepatoblastoma
   - Erythrophagocytic lymphohistiocytosis
10. *Miscellaneous*
    - Neonatal lupus erythematosus
    - "Le foie vide" (infantile hepatic non regenerative disorder)
    - Indian childhood cirrhosis

the right upper quadrant in the case of a choledochal cyst or a characteristic facies in Allagile's syndrome. Growth retardation can be seen with congenital infections, such as rubella and cytomegalovirus.

86.5 Evaluation

Neonatal cholestasis should be urgently evaluated in any infant who is jaundiced after 2 weeks of life [2]. Evaluation should commence with a detailed patient history, including family, pregnancy, antenatal, and postnatal histories, and physical examination. Important clues indicating a cholestatic etiology may be missed without a thorough history and examination. Fractionation of the total bilirubin level is crucial in the initial investigation of cholestasis.

Once cholestasis is established with an elevated conjugated bilirubin, further investigation should proceed in a logical, stepwise manner to ensure conditions requiring immediate intervention, such as galactosemia, sepsis, and hypothyroidism are not overlooked and are treated in a timely manner. Next, evaluation for biliary atresia is crucial as the successful surgical treatment of biliary atresia also depends on timely diagnosis [3]. After biliary atresia has been excluded, evaluation should be done to establish the cause of intrahepatic cholestasis. A suggested approach to the evaluation of infants with cholestatic jaundice is summarized in Fig. 86.1.

86.5.1 Laboratory Evaluations

The first step in diagnosing neonatal cholestasis is the fractionation of the total serum bilirubin. Conjugated hyperbilirubinemia is defined as a conjugated bilirubin greater than 1 mg/dL (17 μmol/L) or more than 20% of the total bilirubin level. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are sensitive indicators of hepatocellular injury but lack specificity or prognostic value. Elevation of alkaline phosphatase (ALP) can be seen in biliary obstruction, but also lacks specificity, as it is also found in bone and kidney. Thus, with ALP elevations, diseases in other organs, such as bone must be excluded. The enzyme gamma glutamyl transpeptidase (GGT) is located in biliary epithelium and elevations are highly sensitive for cholestatic disorders, such as biliary atresia, α_1-AT deficiency, and idiopathic neonatal hepatitis. One exception is seen in progressive familial intrahepatic cholestasis types 1 and 2 in which normal or low levels of GGT can be seen.

86.5.2 Radiographic Investigations

86.5.2.1 Abdominal Ultrasound

Abdominal ultrasonography is a simple, noninvasive procedure that is often used as the first step in the work-up of cholestatic infants. Ultrasound can provide useful information regarding size and appearance of the liver and gallbladder, visualization of gallstones and sludging of bile, and diagnosis of choledochal cysts [4]. The triangular cord sign, which represents a fibrous cone of tissue at the porta hepatis, has been reported to be highly specific and sensitive for biliary atresia, with a specificity of 100% and sensitivity ranging from 85–100% [5, 6]. Abdominal ultrasound can also be used as a screen for anomalies associated with biliary atresia, such as polysplenia and interruption of the inferior vena cava [4].

86.5.2.2 Hepatobiliary Scintigraphy

Hepatobiliary scintigraphy using technetium labeled iminodiacetic acid derivatives represents the only available method of imaging that can evaluate hepatobiliary physiology [7]. As the test can determine the patency of the biliary tract, it is highly sensitive for biliary atresia, but lacks specificity, estimated at 83–100% and 35–50%, respectively [6, 8]. Excretion of the isotope can also be delayed in forms of intrahepatic cholestasis. In biliary atresia, the uptake of the isotope occurs in a normal manner, but excretion into the biliary system and

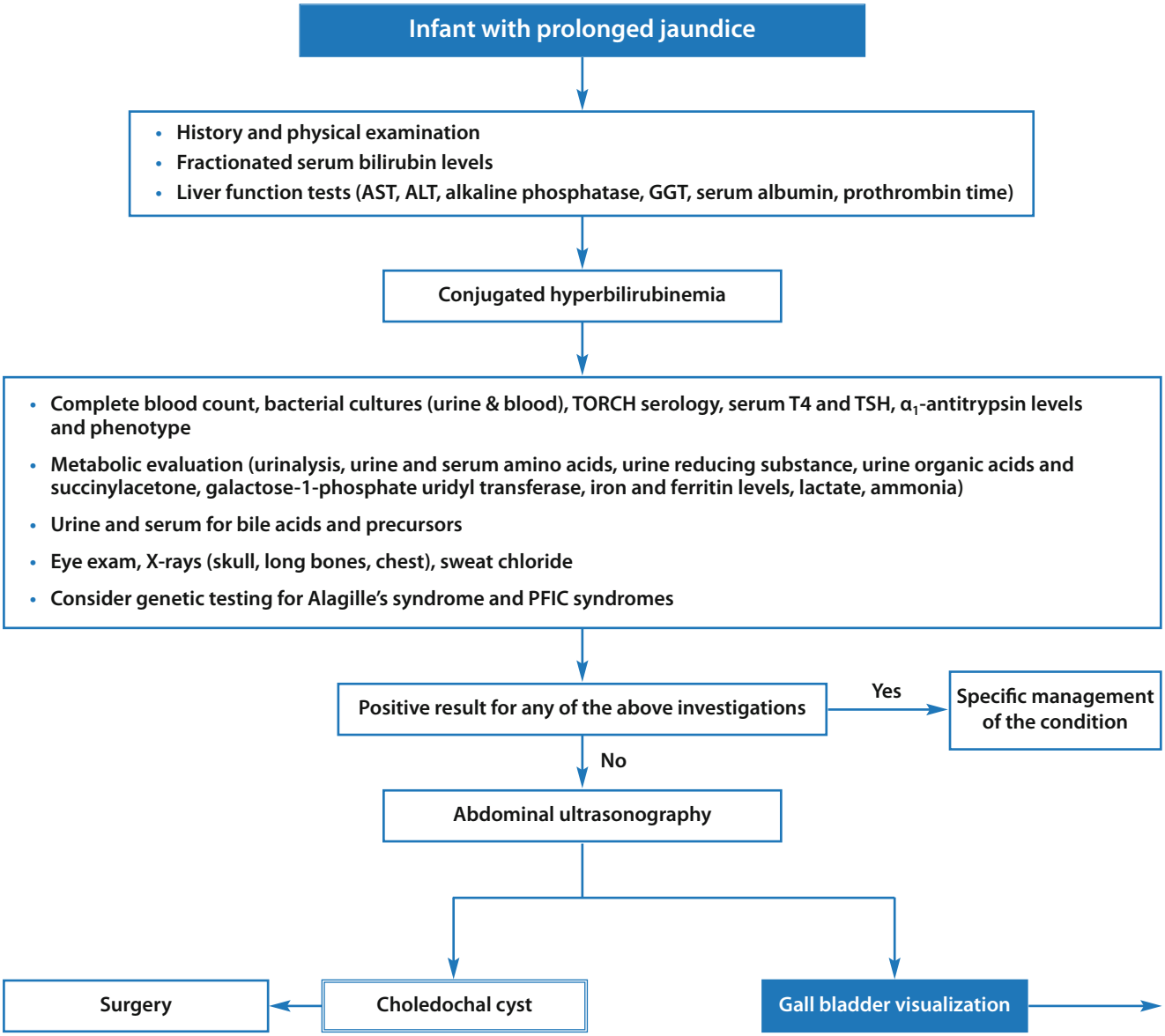
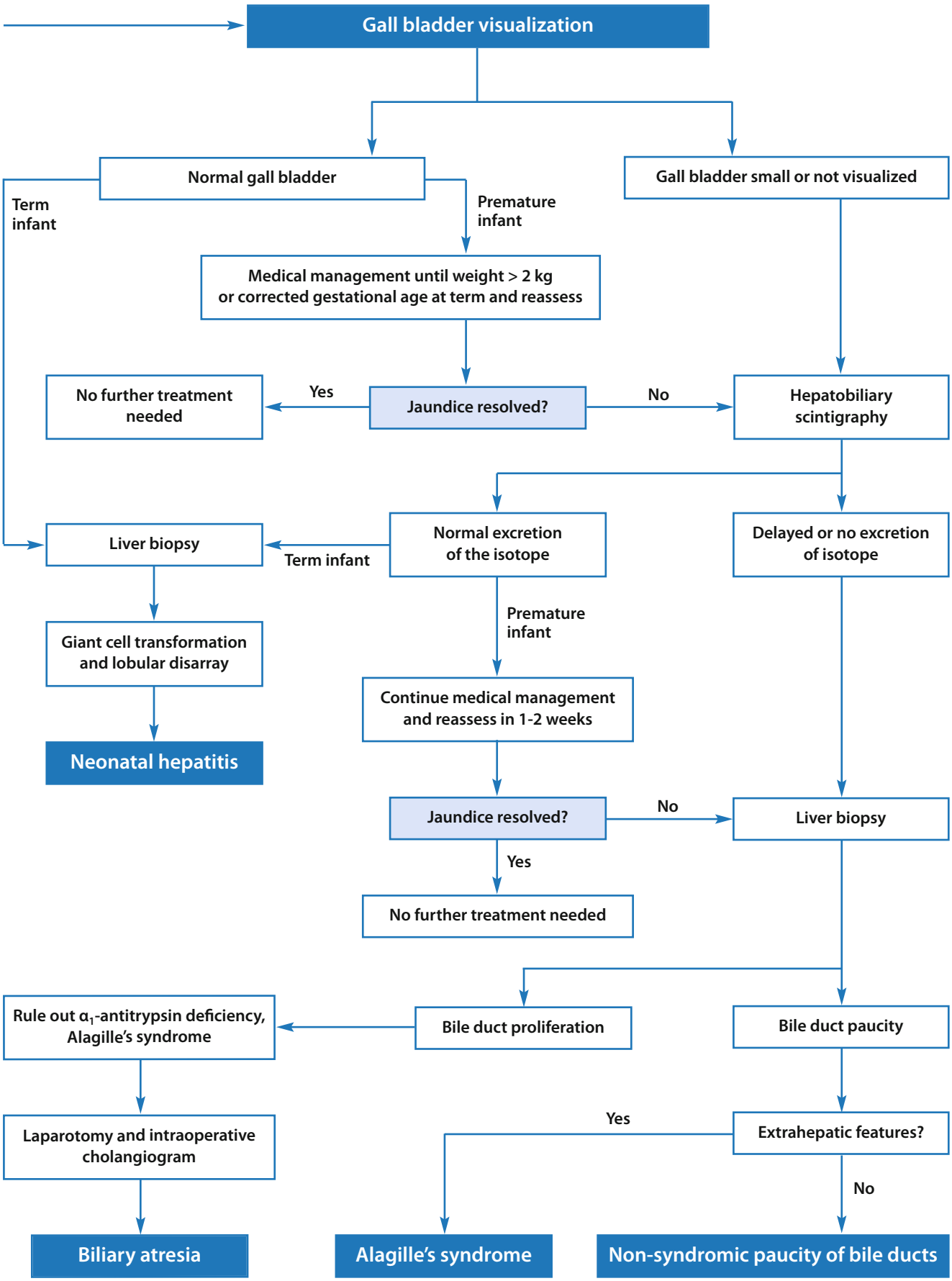


Fig. 86.1 An approach to a full-term or premature infant with cholestasis. Modified from [3], with permission


Gall bladder visualization
Normal gall bladder
Gall bladder small or not visualized
Term infant
Premature infant
Medical management until weight > 2 kg or corrected gestational age at term and reassess
No further treatment needed
Yes
Jaundice resolved?
No
Hepatobiliary scintigraphy
Liver biopsy
Term infant
Normal excretion of the isotope
Delayed or no excretion of isotope
Giant cell transformation and lobular disarray
Premature infant
Neonatal hepatitis
Continue medical management and reassess in 1-2 weeks
Jaundice resolved?
No
Liver biopsy
Yes
No further treatment needed
Rule out α1-antitrypsin deficiency, Alagille's syndrome
Bile duct proliferation
Bile duct paucity
Laparotomy and intraoperative cholangiogram
Yes
Extrahepatic features?
No
Biliary atresia
Alagille's syndrome
Non-syndromic paucity of bile ducts

the intestines, is delayed or absent. In forms of intrahepatic cholestasis, such as in idiopathic neonatal hepatitis, the uptake of the isotope is delayed, but the isotope is ultimately excreted into the intestines. Pretreatment with phenobarbital, 5 mg/kg/day for 5 days, improves sensitivity for biliary atresia by stimulating bile acid-independent flow [1].

86.5.2.3 Magnetic Resonance Cholangiography

Magnetic resonance cholangiography is increasingly used to assess the biliary tract. In biliary atresia, there may be failure to visualize the common bile duct and the gallbladder is small. Sensitivity for detecting biliary atresia has been estimated at 95%, with a specificity of 88% [9]. Further research using this modality is needed to assess its reliability in the evaluation of neonatal cholestasis and excluding biliary atresia.

86.5.3 Duodenal Aspirate Analysis

Duodenal fluid can be obtained by either placement of a naso- or oroduodenal tube or using a string in the duodenum. Bilirubin concentration in the aspirate can then be assessed and a duodenal aspirate bilirubin concentration less than that in the serum is suggestive of biliary obstruction. Although sensitivity of duodenal aspirate analysis approaches that for scintigraphy, the test is more invasive.

86.5.4 Liver Biopsy

Percutaneous liver biopsy is the most definitive test in the evaluation of neonatal cholestasis and should be performed in most infants with undiagnosed cholestasis [2]. Biopsy is recommended prior to a surgical procedure for biliary atresia. If biopsy results are equivocal and the infant is less than 6 weeks of age, biopsy may need to be repeated. Histologic findings in biliary atresia include bile duct proliferation and bile plugs. These findings must be differentiated from idiopathic neonatal hepatitis, in which there is giant cell transformation and focal hepatocellular necrosis. Liver biopsy can also be useful to diagnose congenital viral infection, as viral inclusion bodies suggest cytomegalovirus or herpes infection. Lack of intrahepatic bile ducts in infants less than 1 year of age has been found to be an adverse prognostic factor independent of neonatal liver disease etiology [10].

86.6 Management of Cholestasis

The medical management of cholestasis is largely supportive because the underlying disease is often untreatable. Such treatment addresses complications of chronic cholestasis rather than the underlying cause (Table 86.2). These complications include pruritus, malabsorption, and nutritional deficiencies. See also Chapter 85.

86.6.1 Pruritus

The exact cause of pruritus is unknown but most agree that more than one factor is involved in the pathogenesis of cholestatic pruritus. It is not generally a problem in the care of the newborn. Its causes and treatment have been described in children and adults [11–13], when medical management of cholestatic pruritus is concentrated on decreasing the levels

Table 86.2 Medical and nutritional management of cholestasis

Drug	Dose	Side effects
Ursodeoxycholic acid	10–20 mg/kg/day	Diarrhea, hepatotoxicity
Rifampin	10 mg/kg/day	Hepatotoxicity, drug interactions
Phenobarbital	3–10 mg/kg/day	Sedative effects, behavioral changes
Cholestyramine	0.25–0.5 gm/kg/day	Constipation, steatorrhea; hyperchloremic metabolic acidosis
Vitamin A (Aquasol A)	5000–25,000 IU/day	Hepatotoxicity, hypercalcemia; pseudotumor cerebri
Vitamin D		
Cholecalciferol	2500–5000 IU/day	Hypercalcemia
25-OH cholecalciferol	3–5 mcg/kg/day	Nephrocalcinosis
Vitamin E		
Aquasol E	50–400 IU/day	Potentiation of vitamin K deficiency coagulopathy
TPGS (d-alpha tocopheryl polyethylene glycol-1000 succinate)	15–25 IU/kg/day	Diarrhea Hyperosmolality (with TPGS)
Vitamin K (Phytonadione)	2.5–5 mg every other day	
Water soluble vitamins	Twice the recommended daily allowance	

of circulating bile acids in the blood through a variety of mechanisms.

86.6.2 Nutritional Management

Nutritional management should start at the initial visit and growth parameters including height and weight for age and weight for height measurements closely followed. Decreased bile acid delivery to the intestines is the end result of cholestasis regardless of etiology, resulting in fat and fat-soluble vitamin malabsorption. Malabsorption occurs when the intraluminal bile acids concentration falls below the critical micellar concentration, resulting in decreased long-chain triglyceride lipolysis. Essential fatty acid (EFA) deficiency may occur secondary to malabsorption of long chain triglycerides and inadequate intake. Manifestations of EFA deficiency include growth impairment, dry scaly rash, thrombocytopenia, and impaired immune function [14].

Medium chain triglycerides (MCT) are more readily absorbed and are a better source of fat calories. Cholestatic infants should be started on an MCT-containing formula like Pregestimil or Alimentum, which contain 60% and 50% of fat calories as MCT oil, respectively. As a result of steatorrhea and increased energy expenditure, cholestatic infants should receive approximately 125% of recommended dietary allowance based on ideal body weight. Some infants may need additional calories for catch-up growth if significant malnutrition is present and use of nocturnal enteral feeds may be required if oral intake is not adequate.

The fat soluble vitamins A, D, E, and K require solubilization by bile acids into micelles to be absorbed across the intestines. Cholestatic infants are particularly susceptible to fat soluble vitamin deficiency as a consequence of malabsorption. Doses of at least two to four times the recommended daily allowance are given. Vitamin supplementation should continue at least 3 months after resolution of jaundice. Fat-soluble vitamin levels should be followed during treatment (Table 86.2).

86.7 Specific Diseases

86.7.1 Biliary Atresia

Biliary atresia is a progressive inflammatory cholangiopathy resulting in the obliteration of portions or the entire extrahepatic biliary tree. With time, progressive fibrosis and obliteration of the biliary tree results in biliary cirrhosis. Biliary atresia is the most common indication for pediatric liver transplantation and the etiology is unknown. The incidence of biliary atresia is estimated to be 1:8–15,000 [1]. There are two recognized clinical forms of biliary atresia. The perinatal form is most common, accounting for 80–90% of cases, and presents in the first few weeks of life with the cardinal features of jaundice, acholic stools, and hepatosplenomegaly. In the embryonic form of biliary atresia, infants present with early onset of disease, with jaundice at or shortly after birth. Infants with the embryonic form have associated anomalies that can include vascular anomalies of the splenic or portal veins, hepatic artery, or inferior vena cava. Situs inversus, annular lung disease, and congenital heart disease are additional associated anomalies [15].

The infant with biliary atresia is usually full-term, clinically normal at birth, and thrives without difficulty. With time, jaundice and hepatomegaly develop and stools become acholic. Failure to thrive ensues and pruritus, coagulopathy, and splenomegaly develop as the disease progresses.

86.7.1.1 Evaluation

Thorough history taking and physical exam coupled with a logical, systematic approach will help ensure a timely diagnosis of biliary atresia. After documentation of a conjugated hyperbilirubinemia with fractionation of the total bilirubin, further laboratory testing will show elevated transaminases, alkaline phosphatase levels, and gamma-glutamyl transferase levels. Prothrombin time may be elevated secondary to vitamin K deficiency resulting from fat malabsorption.

Abdominal ultrasound often shows a small or absent gallbladder. Care must be taken during interpretation of ultrasound findings because presence or absence of a gallbladder does not confirm or exclude biliary atresia. Observation of the triangular cord sign, by an experienced ultrasonographer, is highly sensitive and specific for biliary atresia. Splenic and vascular anomalies associated with the embryonic form of biliary atresia may also be seen. If findings on ultrasound are inconclusive for biliary atresia, hepatobiliary scintigraphy should be used to determine the extrahepatic biliary patency. Excretion of isotope into the intestines excludes biliary atresia although lack of excretion into the intestines is not specific for the disease and further evaluation is essential.

Liver biopsy can provide the diagnosis in up to 97% of cases when radiologic studies are inconclusive. In biliary atresia, classic histologic findings include bile duct proliferation, bile plugging, and portal tract edema with periportal fibrosis. As bile ductular inflammation and obliteration in biliary atresia is progressive, if liver biopsy is done early in the disease process, repeat biopsy may be necessary if results are initially equivocal [2, 16].

In the event that diagnosis is not possible after liver biopsy and radiologic evaluation, operative exploration and cholangiography is then recommended. α_1-AT deficiency, as well as other causes of intrahepatic cholestasis, presents similarly to biliary atresia and should be excluded prior to laparotomy, as these conditions can be worsened with portoenterostomy [17].

86.7.1.2 Management

After confirmation of biliary atresia with an intraoperative cholangiogram, the treatment of choice is the Kasai hepatoportoenterostomy. The surgery involves removal of the fibrous tissue at the porta hepatis and formation of a roux-en-Y anastomosis between the duodenum and the hilum of the liver. The two most important prognostic factors determining success of the Kasai procedure are age at operation and experience of the surgeon [18, 19]. The relationship of survival to age is not linear or stepwise and the rate of survival with native liver has been estimated at 90% for those with surgery by 100 days of age compared to 60% if the surgery is performed after 100 days of life [18]. Complications of the Kasai procedure include ascending cholangitis and portal hypertension. Ascending cholangitis is a common and frequently recurrent postoperative complication with affected patients presenting with fever, worsening jaundice, and elevated ESR. Cholangitis can worsen the prognosis in those who have had restoration of bile flow after portenterostomy. Recurrence of cholangitis is the single most significant variable pertaining to long-term prognosis [20]. After initial treatment with intravenous antibiotics, those with recurrent ascending cholangitis may benefit from prophylactic antibiotics, such as oral trimethoprim-sulfamethoxazole or neomycin [21].

Treatment with steroids after the Kasai procedure has been shown to lower postoperative bilirubin levels, but no improvement in terms of cholangitis attacks and early liver transplantation is seen [22]. Portal hypertension may develop in 40–80% of patients within 5 years after the Kasai procedure [15]. These patients have splenomegaly that may be complicated by ascites and variceal bleeding. 20% of the patients that have portal hypertension may be noted to have portal vein thrombosis, likely secondary to ongoing portal tract inflammation. Therapeutic endoscopy with either ligation or sclerotherapy can be used to treat acute variceal bleeding.

In addition to the surgical management, patients should also receive supportive care for cholestasis, including supplementation of fat-soluble vitamins and a high calorie diet. See also Chapter 85.

86.7.2 α_1-Antitrypsin Deficiency

Alpha$_1$-AT deficiency is the most common inherited cause of neonatal cholestasis, caused by mutations in the gene found on chromosome 14. α_1-AT is a glycoprotein with inhibitory activity against elastase, trypsin, and other proteolytic enzymes. More than 75 different variant α_1-AT mutants have been found but only a few are clinically relevant, with M being normal and Z being most deficient. Patients with PiZZ phenotype have greatly reduced amounts of α_1-AT, around 10%–15% of normal, with an incidence of 1 in 2000 live births in European and North American populations [23]. The PiZZ phenotype is associated with neonatal liver disease and adult emphysema.

Approximately 15 percent of PiZZ neonates develop clinical disease within 20 years and accumulation of the defective α_1-AT molecule in the liver is the cause of hepatic injury [24]. These infants have intrauterine growth restriction and are more likely to develop coagulopathy compounding the cholestasis. On biopsy, periodic acid-Schiff-positive diastase-resistant inclusions within hepatocytes represent the abnormal α_1-AT protein. Documenting low plasma α_1-AT levels and determining α_1-AT phenotype confirms the diagnosis.

As with idiopathic neonatal hepatitis, management of α_1-AT deficiency is mostly supportive and includes nutritional supplementation. Prognosis is related to the severity of the liver disease. In children with progressive liver disease, liver transplantation has shown good survival rates of 90 % at 1 year and 80% at 5 years [25].

86.7.3 Idiopathic Neonatal Hepatitis

Idiopathic neonatal hepatitis accounts for approximately 30% of neonatal cholestasis cases. A diagnosis of exclusion, idiopathic neonatal hepatitis is diagnosed by documentation of classic histologic findings on liver biopsy. Although occasional familial cases suggest a genetic association, most cases are sporadic.

Infants with idiopathic neonatal hepatitis are often low birth weight. Jaundice is present within the first few weeks of life and not immediately after birth. The liver may be enlarged on physical examination and serum bilirubin and hepatic transaminases mildly elevated. Low or normal GGT levels may signify more severe disease and a worse prognosis, especially if GGT levels do not increase over the course of the disease [26]. On liver biopsy, findings include lobular disarray with hepatocyte swelling, giant cell transformation, and increased extramedullary hematopoiesis.

In contrast to biliary atresia, management of idiopathic neonatal hepatitis is largely supportive. Prognosis is excellent with 90% resolution by 1 year of age in sporadic cases but worse in familial cases.

86.7.4 Progressive Familial Intrahepatic Cholestasis

Progressive familial intrahepatic cholestasis (PFIC) is a group of genetic disorders, with autosomal recessive inheritance, associated with progressive intrahepatic cholestasis. There are three forms that vary in presentation, diagnosis, and treatment.

PFIC-1, the original Byler disease described in an Amish family, is caused by a mutation in the FIC 1 gene. The FIC 1 gene mutation results in episodic cholestasis in the first month of life. With time, the jaundice resolves but the pruritus continues and end stage liver disease ensues. The FIC1 gene has a wide tissue distribution and besides the canalicular membrane, is also expressed in the pancreas, small bowel, stomach, bladder, heart, and kidney. Such wide tissue expression explains the diarrhea and pancreatitis with fat-soluble vitamin deficiency seen in affected patients. Importantly, serum GGT and transaminase levels are normal, with markedly elevated serum bile salts. Liver histology at biopsy shows bile duct paucity. Management is largely supportive. Surgical approaches that deplete the bile salt pool, such as partial biliary diversion and partial ileal bypass, may be effective. Cirrhosis is usually seen by the end of the 1st decade and liver transplantation for hepatic decompression by the 2nd decade.

PFIC-2 is caused by a defect in the canalicular bile salt excretory pump (BSEP). Clinical presentation is similar to PFIC 1, with the exception of lack of the extraintestinal manifestations. Liver biopsy usually shows more inflammation and management is supportive. Prognosis is worse for those requiring liver transplantation in the 1st decade of life.

PFIC 3 is caused by defective phospholipid flippase function of MDR3, the canalicular phospholipid transporter, resulting in bile characterized by significant concentrations of unmicellized bile salts. Clinical presentation, similar to PFIC 1, is often delayed until early adulthood. There is often a history of cholestasis of pregnancy in the mother. In contrast to PFIC 1 and 2, GGT is markedly elevated and bile analysis shows a high bile acid to phospholipid ratio. Liver biopsy may appear consistent with biliary atresia but the biliary tract will be patent on scintigraphy or cholangiography. The prognosis is variable and, as with the other forms of PFIC, management is supportive.

86.7.5 Cholestasis in Premature Infants

Cholestasis is common in very low birthweight infants, with a multifactorial etiology. The biliary system is immature in these infants resulting in an exaggerated physiologic cholestasis. Other factors can contribute to cholestasis in premature infants, including perinatal hypoxia, sepsis, parenteral nutrition (PN), and enteral feeding difficulties [27, 28].

Cholestasis is a frequent complication of PN. Although the exact etiology is unknown, immaturity of the enterohepatic circulation likely plays a role. Risk factors include early initiation and prolonged PN, lack of enteral feeds, sepsis, and prematurity. Conjugated hyperbilirubinemia with elevated transaminases is usually seen within 2 weeks of commencing PN. Management includes decreasing or cessation of PN and increasing enteral feeds. In those infants who cannot tolerate full enteral feeds, trophic feeds should be instituted to decrease enterohepatic circulation. In such infants, modifications in PN formulation should include limiting glucose intake to 15 gm/kg/day, limiting intralipid, cycling of PN to 12 hours daily, and reducing or eliminating manganese and copper, as these trace nutrients are primarily secreted via bile. Ursodeoxycholic acid can aid in stimulating bile acid excretion via mechanisms previously discussed.

Biliary atresia is rare in premature infants and liver biopsy and hepatobiliary scintigraphy should be delayed in these infants until corrected gestational age (CGA) is term and weight is greater than 2 kg. Liver biopsy should be performed if cholestasis persists past 2 months CGA, presence of acholic stools, and those without excretion on hepatobiliary scan.

86.7.6 Alagille Syndrome

Alagille syndrome is the most common cause of familial intrahepatic cholestasis. Characterized by paucity of intrahepatic bile ducts, Alagille syndrome is inherited in an autosomal dominant fashion, with variable penetrance, and is estimated to affect 1 in 100,000 live births. Mutation occurs in the Jagged 1 gene, on chromosome 20p12, that encodes a protein involved with cell differentiation [29].

Alagille syndrome manifests as chronic cholestasis associated with a characteristic facies and involvement of the skeletal, cardiac, and ocular systems. Infants have a saddle nose and broad forehead. Skeletal system involvement includes butterfly vertebrae, curved phalanges, and short ulna. Peripheral pulmonic stenosis, tetrology of Fallot, pulmonary atresia, and VSD are cardiac anomalies that may be seen. Posterior embryotoxon and optic nerve drusen constitute eye findings. Supernumerary digital flexion creases are seen in 1/3rd of cases [30]. Cutaneous xanthomas secondary to hyperlipidemia can also be seen.

Initially, infants with Alagille syndrome present with neonatal cholestasis, as seen in biliary atresia. Differentiation of the two disorders can be difficult because initial liver biopsies may show bile duct proliferation consistent with BA. Bile ducts are initially structurally normal and are then progressively lost. Characteristic facies may not be evident until 2 years of age.

Management is largely supportive, with nutritional support including fat-soluble vitamin supplementation. Of those who present with cholestasis in the newborn period, over 50% will require liver transplantation by 10 years of age secondary to cirrhosis.

Caution must be used in evaluation of family members as matched living donors, as there may be subclinical disease in such donors. Hepatocellular carcinoma has been reported in patients with Alagille syndrome.

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87

Surgical Treatment of Biliary Tract Malformations

George Ekema and Pierluigi Pedersini

87.1 Biliary Atresia

Biliary atresia (BA) is a progressive necroinflammatory process involving the extrahepatic biliary tree, either partially or completely. The disease shows the onset during the first days of life and usually evolves into obliteration of the extrahepatic bile duct lumen with interruption of bile flow, resulting in cholestasis and chronic liver damage. If untreated, the disease leads to exitus within the first 2 years of life. Early diagnosis is mandatory to plan a timely surgery, preferably within 80 days of life.

Although Kasai portoenterostomy can achieve a good relief of cholestasis in about a half of cases, even children successfully treated show the consequences of liver derangement, namely hepatic fibrosis or cirrhosis with portal hypertension, so that the majority of BA patients need organ replacement later in life. Actually, BA is still the most common indication for pediatric liver transplantation worldwide, and the sequential use of Kasai's procedure and liver transplantation have dramatically increased the survival rate of these patients during the last few decades [2].

BA is the most common cause of cholestatic jaundice in the newborn and its prevalence is about 1:10,000–15,000 live births, meaning approximately 700 newborns affected every year in Europe, with a small female preponderance. Incidence of BA is highest in Asian and Pacific populations and familial cases have been reported [3].

Obliteration of bile ducts can involve any branch of the extrahepatic biliary tree, and the types of BA are classified according to the site of obstruction [4]: type I involves just the common bile duct; type II also involves the cystic and the hepatic ducts and type III, present in more than 95% of cases, involves all extrahepatic biliary ducts and represent the most severe form of BA.

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Two anatomical forms of BA have been identified: nonsyndromic BA (~90%), in which BA is an isolated anomaly; and syndromic BA (~10%), associated with various congenital anomalies such as polysplenia, asplenia, cardiac or intraabdominal defects (situs inversus, preduodenal portal vein, absence of retrohepatic inferior vena cava, intestinal malrotation) [5].

87.1.1 Etiology and Pathogenesis

The origin of BA still remains unclear. Some cases seem to be related to abnormal morphogenesis of bile ducts occurring early in pregnancy (embryonic or fetal BA), while many cases appear to arise from later damage to normally developing bile ducts (perinatal or acquired BA) [5].

Epidemiologic studies support a possible infectous etiology. An association with virus infections has been reported (CMV, RSV, EBV and HPV); in contrast, no association with hepatitis A, B and C viruses has been found [3]. The two viruses most commonly implicated are reovirus and rotavirus. In human neonates the association of Reovirus type 3 and BA has been suggested, but not confirmed otherwise. The role of Rotavirus type C in the etiology of BA in humans remains controversial. Thus, no study so far has managed to definitively prove the role of a specific virus as an etiologic agent of BA.

The role of immune dysfunction has been based on the assumption that the biliary epithelium may express inappropriate antigens on its surface that can be recognized by lymphocytes after viral or toxic damage; an immune cascade will produce inflammation and biliary fibrosis [6].

The progressive nature of liver injury in patients with BA, the presence of lymphocytes in the liver and the association with specific types of HLA, suggest a possible autoimmune, persistent attack against biliary epithelium [7].

Finally, several observations suggest that a genetic component plays a role in the pathogenesis of BA, although this is probably only one of multiple factors.

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87.1.2 Clinical Aspects

The clinical signs are jaundice, acholic stools, dark urine and hepatomegaly. The liver gradually increases in size and consistency along with aging. Splenomegaly also follows hepatomegaly, suggesting portal hypertension. The general condition of the newborn is usually good with adequate weight growth; mild jaundice is often missed and the diagnosis is established later. When the patients are untreated, the majority of them will die of hepatic failure, esophageal variceal bleeding and infection [1].

87.1.3 Diagnosis

Antenatal diagnosis of BA remains exceptional. Since early diagnosis appears essential for successful surgical treatment, neonatal jaundice lasting more than two weeks should be investigated for BA

There are many causes of pathological jaundice in the neonatal period and the most likely to be confused with BA are neonatal hepatitis syndrome and interlobular biliary hypoplasia. When jaundiced infants show acholic stools, dark urine and hepatomegaly with hardening in consistence, BA should be strongly suspected [1].

Biochemical liver function tests show increased total bilirubin (TB) with predominance of direct bilirubin (DB) of more than 50% and high levels of γGT, ALT and AST (more than 2–3 times normal values). BA is very strongly suspected with the presence of TB greater than 4.5 mg/dL, DB more than 80% of TB and γGT greater than 500 UI/L [1].

Ultrasonography of the liver may identify a small, nondistended gallbladder and show the "triangular chord sign", representing the fibrous cone of bile duct remnant at porta hepatis [8]. This sign demonstrates high specificity and positive predictive value of 95% for the diagnosis of BA [9]. Ultrasonography also plays an important role in the assessment of splenomegaly or associated anomalies, such as polysplenia.

Hepatobiliary scintigraphy (e.g., Tc-99m DISIDA) shows a failure of escretion of the radioisotope into intestine, but this feature can also be observed in any severe neonatal cholestasis and therefore it is of limited value. The role of endoscopic retrograde cholangiopancreatography (ERCP) [10] and Magnetic Resonance Cholangiography (MRC) [11] in the diagnosis of BA have still been under discussion, because they may be performed in selected skilled centers only.

The definitive diagnosis is based on the fibrosing obstruction of the extrahepatic biliary tree during exploratory laparotomy with cholangiography and liver biopsy [1]. The main histological features suggestive of BA are bile plugs, ductular proliferation, inflammatory infiltration and portal fibrosis [12].

87.1.4 Therapy and Treatment

The current surgical management of BA provides two steps: Kasai operation in neonatal period, which aims to restore bile flow; and later liver transplantation, when the Kasai operation has failed [3].

The Kasai operation is a portoenterostomy, introduced by Kasay and Suzuky in 1959, in which biliary drainage is established by a 45 cm Roux-en-Y loop anastomosis to the hepatic hilum, after careful dissection to locate patent bile duct remnants in the porta hepatis [19]. An alternative laparoscopic approach was first reported in 2002 [13].

The best surgical results after Kasai operation are obtained in children operated within 60 days of life, with satisfactory biliary drainage in at least 70–80% of cases [5], resulting in increased pigmentation of stools and resolution of jaundice. Thus, the age at treatment is one of the most important factors determining surgical outcome, that can however be influenced by postoperative complications.

The most common complications following the Kasai operation are cholangitis and portal hypertension. Rare complications include hepatopulmonary syndrome, pulmonary hypertension, intrahepatic biliary cavities and malignancy [3].

Cholangitis occurs particularly in the first week or months after the operation in 40–60% of cases and is characterized clinically by fever, irritability, vomiting, jaundice, choluria and acholic stools; treatment requires IV antibiotics and corticosteroids [21]. Portal hypertension occurs in at least two-third of children after Kasai operation and the most common sites of varices include esophagus, stomach and Roux loop. Treatment requires variceal sclerotherapy or band ligation; severe hypersplenism may exceptionally require splenic embolization [3].

Factors shown to predict outcome after Kasai operation include: age at operation, experience of the surgeon, site of atresia of extrahepatic bile ducts and number and severity of episodes of cholangitis [5, 14].

87.1.5 Prognosis

Overall, Kasai operation is curative in approximately 20% of patients, while the other patients develop progressive hepatic failure that leads to liver transplantation [1, 2].

The choice of sequential treatment by Kasai operation and secondary liver transplantation is supported by the following reasons: firstly, performing liver transplantation in all children with BA would deprive some of these of the possibility to live with their own liver, while the long-term results of liver transplantation and prolonged immunosuppression are not entirely known; secondly, performing transplantation on all children with BA will dramatically increase the need of pediatric liver grafts at this time when the shortage of organs is as yet an

unsolved problem; lastly, liver transplantation at an older age reduces the period of immunosuppressive therapy.

The overall prognosis of BA has improved since the early days of pediatric liver transplantation. Currently, about 90% of children with BA, treated by Kasai operation and liver transplantation have an acceptable quality of life [3, 14].

87.2 Congenital Bile Duct Dilatation

Congenital bile duct dilatation (CBDD) is a relatively rare malformation of the pancreatobiliary system with an incidence between 1:130,000 and 1:2,000,000 and a female predominance (F:M = 3:1). For unknown reasons, it is much more common in Asians, with the majority of the reported cases in Japan. A maternally inherited condition, such as an X-linked dominant trait, has been suggested recently. Several cases of familial recurrence have been reported [15].

In 1977, Todani classified CBDD into 5 types:

- Type I: cystic dilatation of the common bile duct (80% of cases)
- Type II: diverticulum of the common bile duct (10%)
- Type III: choledochocele (4%)
- Type IV (11%)
  a. intra-extrahepatic bile ducts dilatation
  b. plurisegmental common bile duct dilatation and
- Type V: isolated intrahepatic bile ducts dilatation (<1%).

87.2.1 Etiology and Pathogenesis

The etiology of CBDD remains essentially unclear. An anomalous pancreatobiliary duct junction with a long common channel involving the common bile duct and the main pancreatic duct is often present and may be a responsible factor for CBDD, allowing pancreatic reflux into the biliary system and leading to duct wall destruction and dilatation. Distal common bile duct stenosis or obstruction may add to ductal dilatation. An autonomic dysfunction by diminished ganglion cells in the distal common bile duct causing partial obstruction and proximal dilatation has been suggested.

87.2.2 Pathology

Two histopathological types of CBDD that may be present concurrently or separately have been described: the "glandular" type, in which there are microscopic cavities in the mucosal layer with chronic inflammatory infiltrate; and the "fibrotic" type, in which the wall is composed mainly of fibrous tissue with interrupted elastic fibers, well-developed collagen fibers and lower inflammation.

87.2.3 Clinical Aspects

It is primarily a disease of older infants and young children and more than half of cases present in the first decade of life. The classic presentation of CBDD with jaundice, abdominal pain and a right hypocondrial mass is seen only in one third of children. Infants usually present with asymptomatic jaundice that may be mild and intermittent or progressive due to complete biliary obstruction. Late complications in cases with delayed or escaped diagnosis are represented by cholelithiasis, cirrhosis, portal hypertension, liver abscess, spontaneous rupture and biliary carcinoma [16].

87.2.4 Diagnosis

There are no specific laboratory tests, and the initial diagnosis is made by US. The findings of a dilated common bile duct with US is helpful for early diagnosis of CBDD. TC may show the cystic dilatation better than US, it does not however demonstrate ductal anatomy [17]. Because biliary duct imaging is essential, the diagnosis must be confirmed by MRC, especially when dealing with the fusiform type [18]. ERCP requires an experienced endoscopist and must be done in older infants or young children with caution, in order to avoid infection of the cyst or precipitate pancreatitis. In most cases, CBDD have been demonstrated in the developing fetus by antenatal maternal ultrasound.

87.2.5 Therapy and Treatment

In the past, internal drainage by cistoenterostomy was the accepted surgical treatment. Nevertheless, this procedure led to significant morbidity due to recurrent cholangitis, cholelitiasis, pancreatitis, anastomotic stricture and persistent risk of biliary carcinoma. The surgical procedure of choice today is cholecistectomy and total excision of the CBDD with a Roux-en-Y hepaticojejunostomy. Before intervention, a cholangiography through the gallbladder is performed to accurately demonstrate the anatomy of the cyst and the pancreatobiliary ductal system.

When the ductal dilatation extend into the main hepatic ducts, after excision of all involved ductal tissue, polyductal Roux-en-Y jejunostomy must be performed. In case of choledococele, which is located inaccessibly within the distal common bile duct, complete surgical excision is precluded. The approach should be transduodenally, and the presenting wall of choledococele is excised with marsupialization of choledococele into the duodenum.

The laparoscopic excision of CBDD is an alternative to standard surgical approaches and the first large pediatric series was published in 2004 [19]. This approach is technically feasible, safe and effective with a comparable outcome to the

open procedure. Additional benefits of laparoscopic approach, compared to laparotomy, are less postoperative pain with less pulmonary complications, a shortened period of ileus and less adhesion formation [20]. The increasing number of laparoscopic biliary reconstruction suggest that this procedure should be considered as a viable alternative to standard surgical approaches, nevertheless it is technically demanding and requires advanced skills; a long-term follow-up is still required to clearly demonstrate their benefits [20].

87.2.6 Prognosis

If the dilated bile duct is totally excised and surgery is performed prior to irreversible liver damage, the prognosis is excellent. Currently, the hepaticojejunostomy has a low surgical mortality and morbidity rate. Cholangitis, anastomotic stricture, and pancreatic duct injury are main postoperative complications.

87.3 Congenital Solitary Liver Cyst

Solitary liver cyst (SLC) is a benign lesion seen four times more often in females and it can be classified as simple solitary liver cyst and solitary intrahepatic biliary cyst [21]. The spectrum of potential pathologies causing congenital solitary liver cysts in children is wide [22].

87.3.1 Etiology and Pathogenesis

The etiology of simple congenital cysts is unknown. They are generally thought to develop from aberrant bile ducts or as a result of a vascular disruption during the fetal period [21].

87.3.2 Pathology

Simple cysts are typically unilocular, lined by cuboidal or columnar epithelium and underlined by loose connective tissue. The cystic fluid may be clear, brown or, occasionally, bilious [23]. They rarely communicate with the biliary tree and are not considered premalignant. They may be completely intrahepatic, partially extrahepatic, or pedunculated. The presence of septa is usually regarded as a pointer to other pathologies and the need for further investigations [22].

87.3.3 Clinical Aspects

Most solitary liver cysts are asymptomatic and identified incidentally or antenatally in the third trimester [21, 24]. Simple cysts rarely cause symptoms unless large. In such cases abdominal distension, feeding difficulties, respiratory distress and duodenal obstruction have been reported [25]. Infection, hemorrhage or rupture are other rare complications.

87.3.4 Diagnosis

US and TC are widely used for the diagnosis of SLC; US should demonstrate the cyst location, loculation, wall contour and characteristics, content and associated pathology. In the differential diagnosis, TC and MRI should be able to show a mesenchymal hamartoma [24]. In selected cases, functional hepatic scintigraphy may be useful in distinguishing a simple from choledochal cyst by the different radioisotope uptake [22].

The differential diagnosis of upper quadrant abdominal cysts and masses include hepatobiliary lesions such liver cysts, parasitic cysts, benign tumors (mesenchymal hamartoma, cystadenoma), malignant tumors (sarcoma), choledochal cyst and biliary atresia; other intra-abdominal cysts (ovarian, omental, mesenteric, adrenal or renal cysts) and different conditions such as dilated bowel loops, duodenum and gallbladder duplications [26].

87.3.5 Therapy and Treatment

Most unilocular cysts do not require intervention postnatally. They remain asymptomatic and may even decrease in size over time. Intervention is required among children who are symptomatic for a mass effect [24]. Percutaneous aspiration has been reported antenatally and postnatally and is a possible alternative to open surgery. The total excision or enucleation seems to be the treatment of choice, but will depend on the anatomical lesion. If this it not possible, partial excision with marsupialization of the cyst wall is recommended [21, 25].

During surgery, communication with the extrahepatic and intrahepatic biliary sistem has to be ruled out by cholangiography [21]. In the case of a biliary cyst, a Roux-en-Y cystoenterostomy or hepaticojejunostomy as described for CBDD, is recommended [23]. Radical liver resection should be avoided to minimize postoperative complications.

87.3.6 Prognosis

The postoperative course can be complicated by bile leakage, cholangitis, septicemia and recurrence of the cyst [21, 22]. Children who undergo partial excision must be followed up closely for recurrence.

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88

Orofacial Malformations

Roberto Brusati and Giacomo Colletti

88.1 Embryology

During the 4th week of intrauterine life, a central depression forms at the front of the cephalic portion of the embryo, i.e., the primary stomodeum, around which are protuberances. The fronto-nasal protuberance is at the top (the future front part of the brain), the mandibular part of the 1st branchial arch is below, and the maxillary protuberances (also derived from 1st branchial arch) are on the sides. Two ectodermal thickenings (olfactory pits) form on the lower surface of the nasal-frontal protuberance: external and internal nasal processes. Meanwhile the maxillary processes grow forward and medially, meeting and fusing with the nasal processes and forming the upper lip and nostrils. Simultaneously within the floor of the primitive stomodeum, the tongue forms from the merging of five processes. The palatal processes form on the sides and a median laminar structure on the roof will become the septum.

At about the 5th week of gestation, the maxillary and mandibular processes merge, forming the ear, the temporo-mandibular joint and the muscles of mastication. The tragus, root of the helix and antihelical fold originate from the 1st branchial arch while the helix, antihelix, antitragus and lobe derive from the 2nd branchial arch.

At the 8th week of gestation, palatal processes (previously arranged vertically on the sides of the tongue) rise and become horizontal and then meet the septum in the midline to separate the oral from the nasal cavity.

88.2 Cleft Lip and Palate

Cleft lip and palate is one of the most frequent congenital malformations: frequency around 1 in 800 births. 20–30% of cases have a genetic basis while in others there is a teratogenic factor, e.g., a viral or bacterial infection, or the use of medications (e.g., cortisone, phenytoin, high doses of vitamin A), a hypovitaminosis (folic acid), alcohol or smoking. These factors have to act during the first two months of gestation for teratogenicity to occur and the genetic backgrounds on which these factors act is crucial for the teratogenic effect.

A cleft lip can be diagnosed from 12 weeks of intrauterine life by ultrasound while the cleft palate is better diagnosed by MRI. There are different anatomical types. The lesions may be:

a. unilateral or bilateral;
b. form where only the lip is affected, which are considered complete if the involvement goes from the nose floor to the vermilion, and incomplete if only the lower part of the lips is affected;

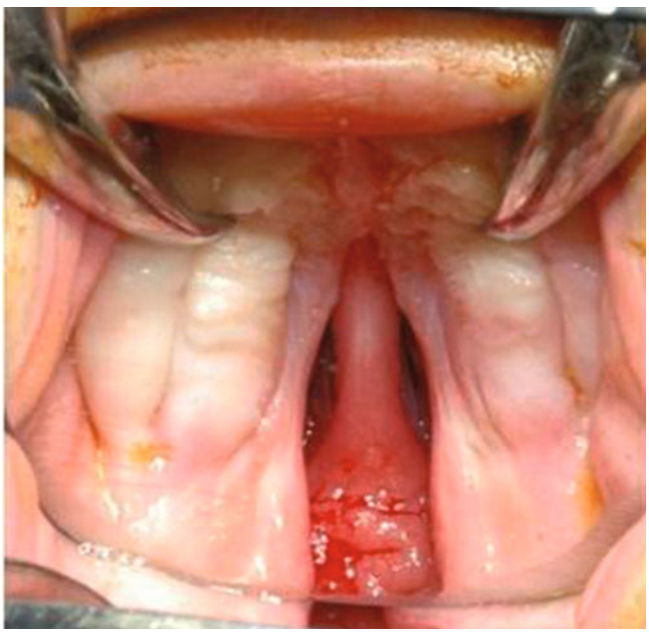

Fig. 88.1 Cleft of hard and soft palate (CP)

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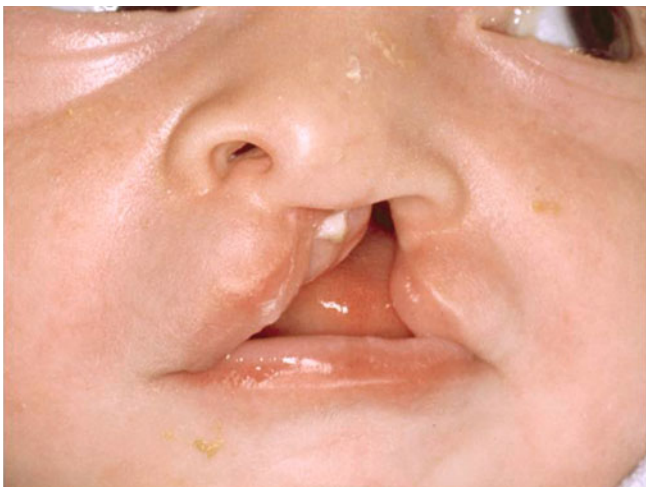

Fig. 88.2 Unilateral complete cleft lip and palate (CLP)

c. form affecting only the palate, involving only the soft or soft and hard parts or consisting of a failure of muscle fusion (submucous cleft) (Fig. 88.1);
d. form affecting the lip, alveolus, maxilla, hard and soft palate (complete cleft lip and palate) (Fig. 88.2).

At birth, children with cleft lip alone present only a cosmetic problem, whereas cleft lip and palate or isolated cleft palate are characterized by an impaired ability to suckle. Feeding is enabled by use of a nipple with an enlarged hole and a plastic bottle that can be gently squeezed (the ability to swallow is normally preserved) and even passage of milk from the nose is without serious consequence. A nasogastric tube should then be avoided. Treatment requires collaboration between different specialists: surgeon, orthodontist, otolaryngologist, pediatrician, psychologist and speech therapist.

In very wide clefts, when there is the interposition of the tongue between the stumps, a palatal plate is applied from the first days (Fig. 88.3). This can reduce the cleft size and facilitate feeding. In bilateral forms, where there is a very short columella, the insertion of a palatal plate with two intranasal prongs (Fig. 88.4) is associated with preoperative lengthening of the columella [1].

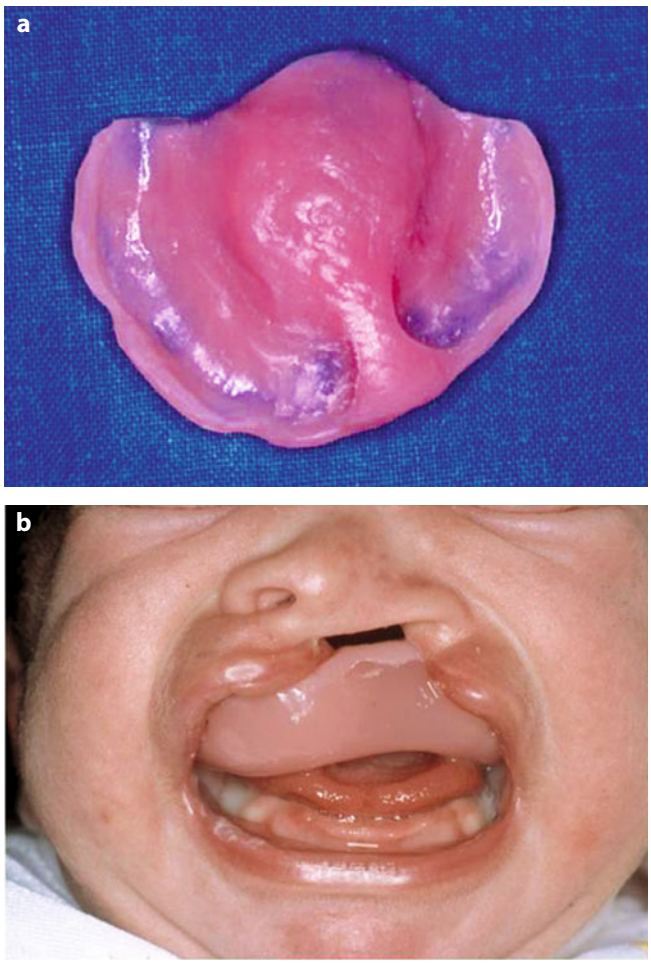

Fig. 88.3 Orthopaedic plate (**a**) in place (**b**)

There are various surgical approaches. At the maxillo-facial unit at University Hospital San Paolo, Milan, Italy, the soft palate is generally repaired at around 4–6 months [2–4] together with lip and nose. The hard palate and the alveolar region (gingivo-alveolo-plasty) are repaired between 18–36 months. In unilateral cases less than 10 mm in width, the lip, nose, and hard and soft palate are repaired in a single operation

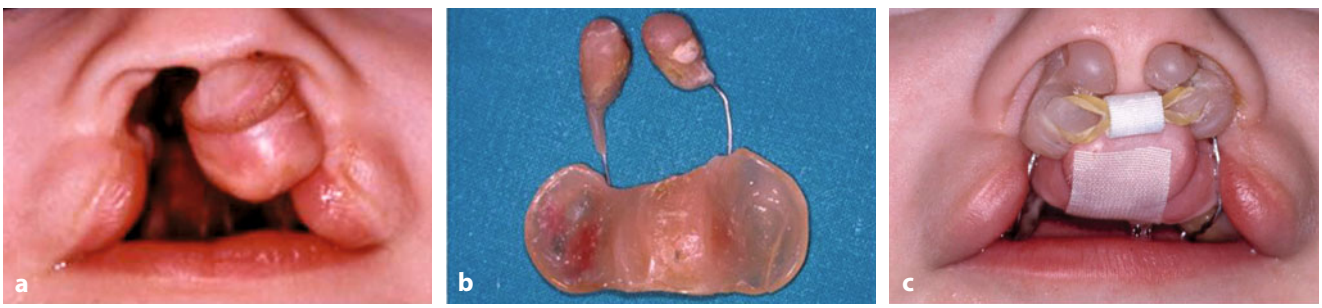

Fig. 88.4 **a** Bilateral CLP with a very short columella. **b** Orthopaedic plate to lengthen the columella. **c** The plate in place and the lengthened columella

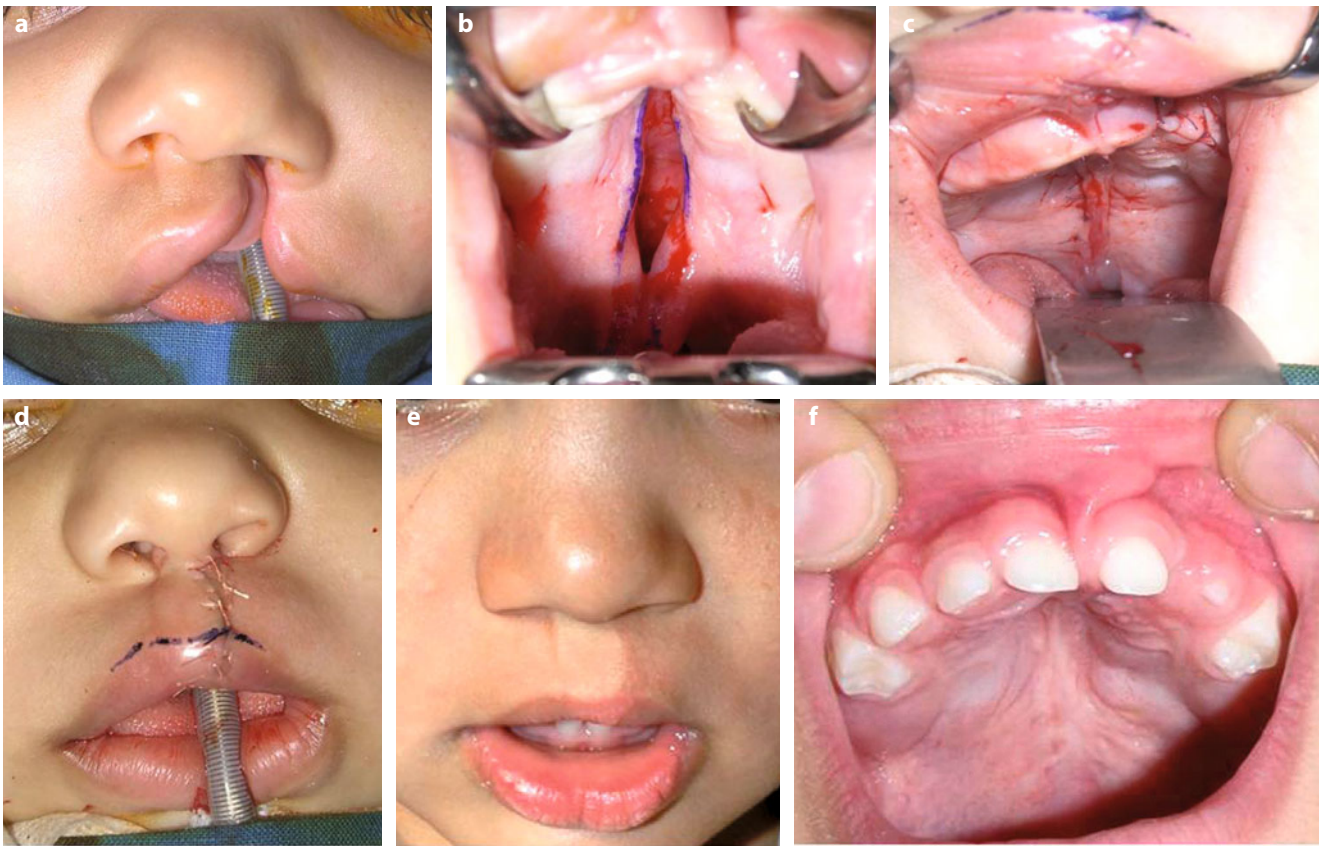

Fig. 88.5 Unilateral CLP operated "All in one"

(Fig. 88.5) [5]. When there is no early repair of the alveolar region, bone grafting is done at 6 years. These children are at high risk of developing ear infections because of altered function of the tensor palati muscle, which has its insertion at the auditory tube, and the otolaryngologist assesses the condition of the ears and the possibility of placing a trans-tympanic drain at the first procedure.

The orthodontist corrects malocclusions that are often present, generally not before the age of mixed dentition. The speech therapist evaluates phonation at 3 years. In a small number of cases, further work may be necessary to improve velopharyngeal competence. In some cases (20%), a maxillary osteotomy is required at the end of growth to optimize appearances and the occlusion with possible adjustments of residual nasal and labial anomalies.

88.3 Pierre Robin Sequence

Pierre Robin sequence is characterized by micrognathia, cleft palate, and glossoptosis. This sequence, which has a frequency between 1/10,000 and 1/20,000 newborns, is due to micrognathia that does not allow the tongue to go down to

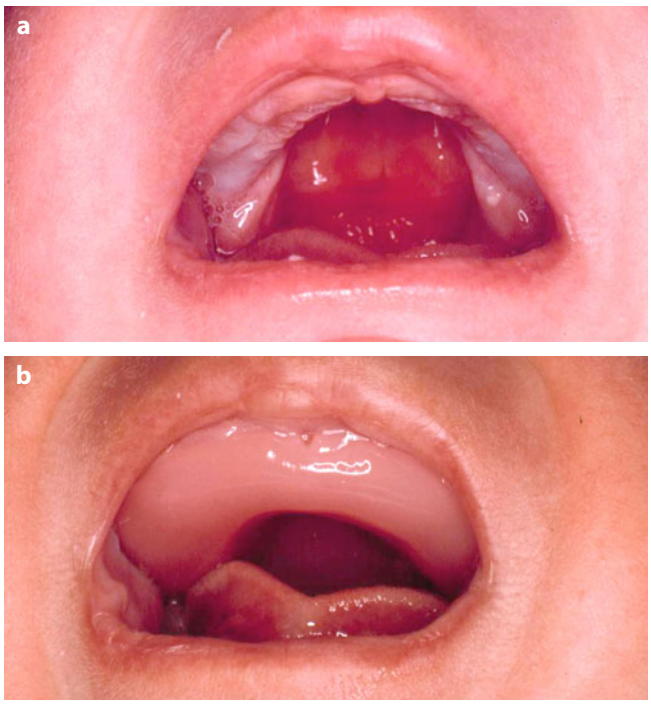

Fig. 88.6 Pierre Robin sequence: **a** typical extremely wide cleft palate; **b** orthopaedic plate in place

the floor of the mouth, remaining in the nasal cavity and preventing the palatine plates from rising and meeting and subsequently fusing in the midline. The result is a glossoptosis that may cause serious respiratory problems at birth. This may be associated with delayed neurological maturation and reduced tone of the muscles.

Treatment at birth ranges from simple positioning of the baby (lying on the side or prone) to the application of a palatal plate to prevent the tongue catching in the wide cleft palate (Fig. 88.6). A nasopharyngeal tube may be required for a few days since muscle tone often improves with the neurological maturation and problems related to glossoptosis resolve. If further intervention is required, the surgical procedure widely employed with success is the so-called tongue-lip adhesion (tip of the tongue to the internal surface of lower lip) (Fig. 88.7).

In severe cases, osteodistraction may be performed to stretch the microgenic jaw [6]; the tongue follows the chin forward with enlargement of the respiratory space and resolution of breathing difficulties.

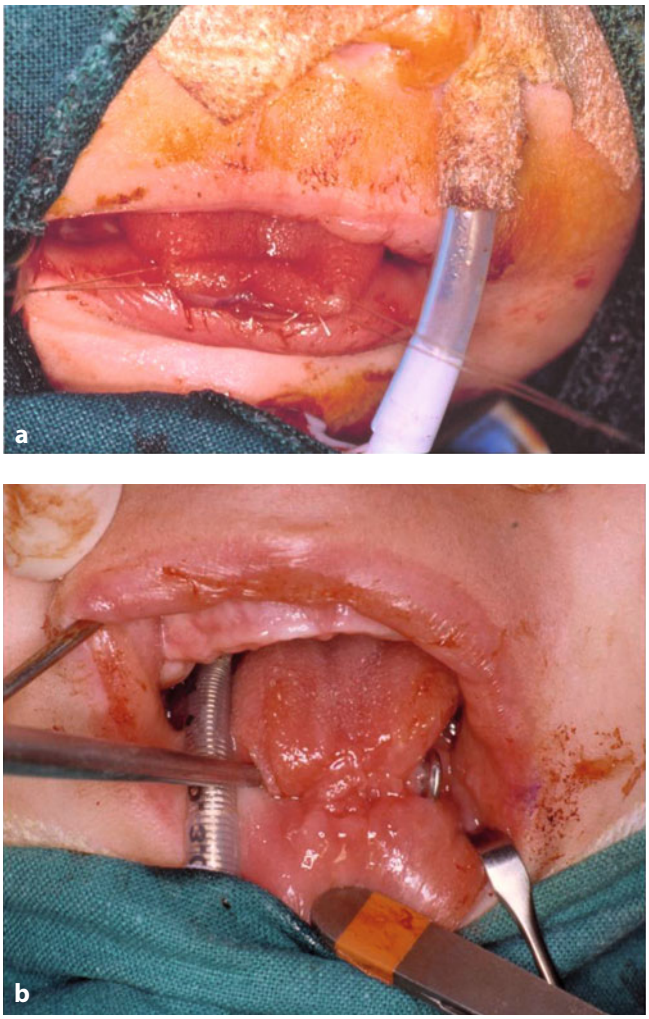

Fig. 88.7 Pierre Robin sequence treated by lip-tongue adhesion: **a** some months later; **b** at release

88.4 Hemifacial Microsomias

In this chapter we will consider congenital malformations that arise with a common etiopathogenetic mechanism: a lesion of the 1st and 2nd branchial arch, including oto-mandibular dysostosis, Goldenhar syndrome and the Franceschetti syndrome.

88.4.1 Etiology and Epidemiology

Bleeding from the stapedia artery caused by teratogenic factors during the 30–40th day of gestation causes tissue disruption at the 1st and 2nd branchial arch. Oto-mandibular dysostosis, an autosomal dominant in a small percentage of cases (2%), affects one in every 5000 newborns. Franceschetti syndrome, a low penetrance autosomal condition with a negative family history in 50% of cases, affects one in every 50,000 babies.

88.4.2 Oto-Mandibular Dysostosis

There are different clinical types of oto-mandibular dysostosis. Microtia occurs in the most serious forms. There is absence of the pavilion (the cartiliginous part of the outer ear), meatus, middle ear, and temporo-mandibular joint; hypoplasia or absence of masticatory muscle; unilateral macrostomia, hypoplasia of the zygomatic arch, pre-auricular fibrocartilaginous appendices; and partial impairment of facial nerve. The consequence of those malformations is chin deviation toward the affected side and malocclusion. Incomplete forms may affect only the ear or the jaw and milder forms may slightly affect one or all of the components. When the jaw is involved, forms range from mild (where the condyle is hypoplastic and mandibular deviation is small [Type I]), to the most serious forms, where the condyle or the whole ramus are totally absent (Type III).

Surgical treatment varies according to the severity and the anatomical region involved. The pavilion may be reconstructed using autologous rib cartilage in two surgical operations carried out at intervals of 6 months at 10 years of age. The jaw is reconstructed (if absent) at around 5–6 years with a osteo-cartilagineous transplant from a rib that serves as a growth center. For defects of moderate severity, surgery can be performed at 5–6 years using osteodistraction. However, since a growth deficit is possible, a final correction is necessary after puberty [7].

88.4.3 Goldenhar Syndrome

The Goldenhar syndrome is basically similar to oto-mandibular dysostosis (with less mandibular involvement) with additional

ocular malformations (epibulbar cysts, lipomas, coloboma of the upper eyelid), vertebral and rib malformations. Malformations may be bilateral but asymmetric. The treatment is similar to that of oto-mandibular dysostosis but early treatment of eye malformations is required if they cause functional problems.

88.4.4 Franceschetti Syndrome

Franceschetti syndrome is characterized by bilateral and symmetrical malformations. There is a severe hypoplasia or aplasia of the cheekbone with the absence of the lateral floor of the orbit and of the corresponding orbital margin to the zygomatic process of the frontal bone and absence of the zygomatic arch. There may be a coloboma on the medial side of the lower lid with absent cilia. The mouth is wide and there may be involvement of the ear (the pavilion, the middle and inner ear). The mandible is severely hypoplastic with a receding chin. There may be other associated malformations such as cleft palate and vertebral anomalies.

Osteodistraction of the mandible can be performed during the first months of life if there are breathing problems. The correction of orbital problems (requiring an autologous bone graft) is performed at around 10 years and the jaws are repaired by bimaxillary osteotomies and genioplasty.

88.5 Craniostenosis and Craniofaciostenosis Malformations

Craniostenosis are related either to altered growth of the sutures or synchondrosis of anterior cranial base. The growth of sutures is influenced by an increase on the size of the skull contents. However, synchondrosis has an intrinsic growth potentiality and may therefore be considered as a real growth center. In this case recent molecular biology and genetics studies have shown inactivation of fibroblast growth factors receptors (FGFRs). The incidence of these malformations is 1/2000 for trigonocephaly, 1/10,000 for plagiocephaly and 1/65,000 for Apert's disease. They are most frequently sporadic, although there are some cases with autosomal dominant or recessive transmission. The following are the most frequent forms.

88.5.1 Trigonocephaly

The premature fusion of the metopic suture determines the characteristic shape of the forehead as a ship's prow. There is reduced transverse growth (with a short inter-temporal distance); there is usually also hypotelorism (reduced distance between the orbits). Surgical treatment is done at 3 months for more severe forms and between 6 and 12 months for moderate forms. Surgery consists of removal of a wide forehead bone flap and of the upper orbital frame, which is remodeled and reintegrated with resorbable fixation materials. The forehead flap is re-fashioned to restore normal facial appearances. The surgery is extradural and needs to be carried out by a collaboration between maxillofacial and neurosurgeons.

88.5.2 Plagiocephaly

This malformation is characterized by skull and face asymmetry and it is determined by the early fusion of half of the coronal suture. As with other cases of partial synostosis, there are no neurological sequelae due to intracranial hypertension. The face is characterized by backwards displacement of the superior orbit and frontal region on the affected side and hypoplasia of the cheekbone. The nose may be deviated towards the unaffected side and the ear of the affected side is in a forward position compared with that of the opposite side. Surgery may be performed in very severe cases at between 6 and 12 months: the upper frame of the orbit and the forehead are relocated to allow symmetric facial growth and an improved cosmetic result.

88.5.3 Scaphocephaly

There is premature fusion of the sagittal suture, resulting in compensatory growth of the coronal and lambdoid sutures and an elongated appearance of the skull. In less severe forms it is difficult to decide clinically if there is dolicocephaly or mild scaphocephaly. In the case of scaphocephaly, 3D CT shows a fused sagittal suture, and treatment, if indicated, is done at around 3 months. It comprises craniectomy (may be by endoscopy) with two paramedian craniectomies on the side of the sagittal suture, another parallel to the coronal suture and another at the level of the lambdoid suture (Fig. 88.8).

The intention is to free brain growth, expand the temporoparietal region and allow the forehead and occiput backwards and forward, respectively. If surgery is postponed it becomes more invasive and difficult and requires complete disassembly and reassembly of the skull.

88.5.4 Craniofaciostenosis

Includes Crouzon's and Apert disease. Coronal synostosis, syncondrosis and synostosis of the bones of the anterior cranial fossa are sometimes associated with lambdoid synostosis (particularly in Apert disease) and involve a serious alteration of facial growth. The reduced anteroposterior growth of the anterior cranial fossa and of the orbital frame together with maxillary underdevelopment lead to a reduced depth of the

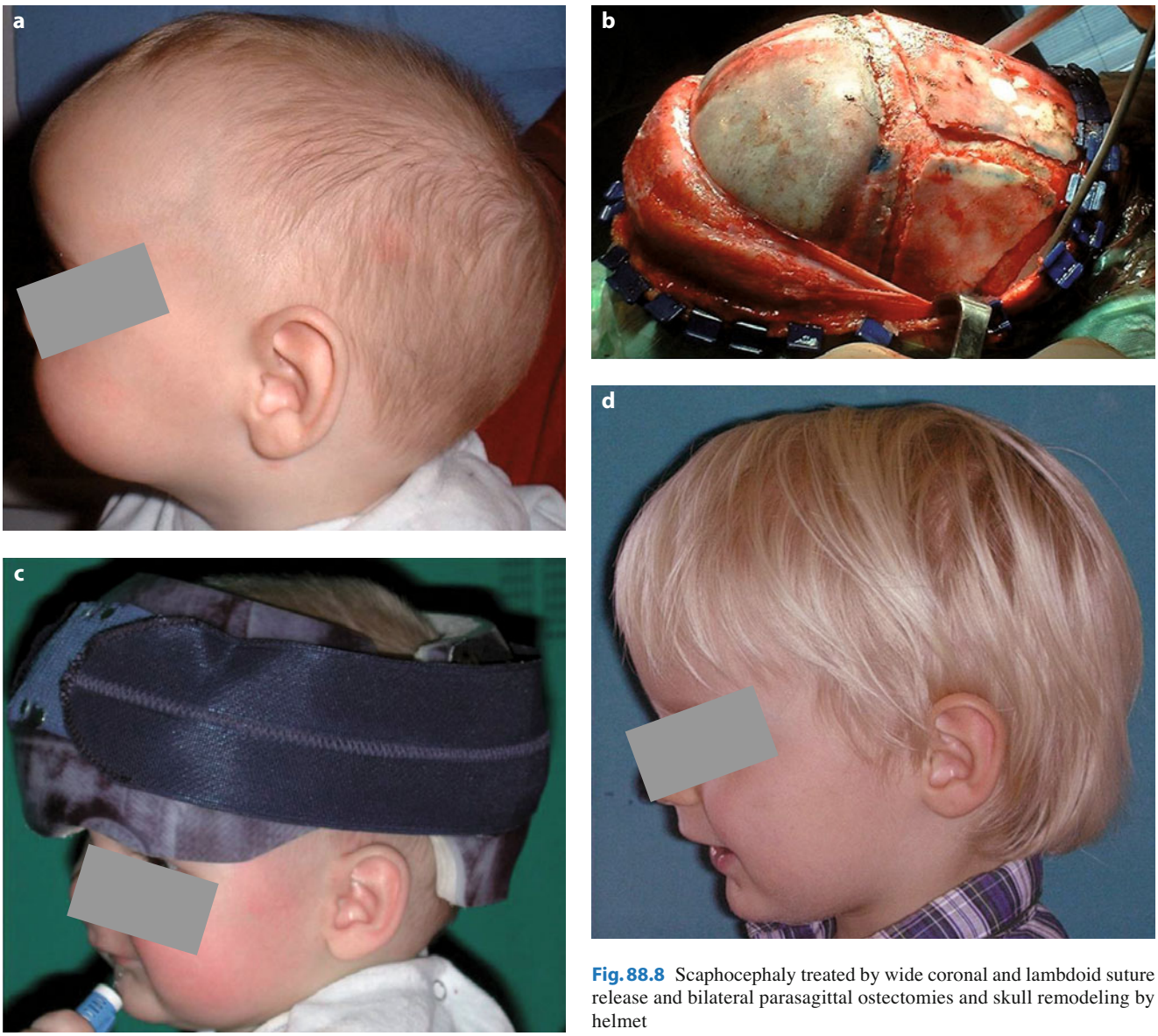

Fig. 88.8 Scaphocephaly treated by wide coronal and lambdoid suture release and bilateral parasagittal ostectomies and skull remodeling by helmet

orbit. This causes the most visible symptom of the disease, exorbitism (normal ocular volume in a small orbit). Besides the serious consequences of lack of growth on the brain, there may be ocular and respiratory problems. The range is from stasis of the papilla to corneal ulcers, causing severe eye infections and corneal lesions and diplopia. The respiratory problems include sleep apnea, which is secondary to upper jaw underdevelopment, and possible cardiopulmonary consequences. There is also an abnormal dental occlusion (open bite). In Apert's disease there is also cleft palate and syndactyly.

Treatment generally separates the neurosurgical from the facial stage. During the first months of life, the orbit and the forehead are brought forward. At 6 years, osteotomy (Le Fort type III) and distraction are performed. If exorbitism is associated with severe breathing problems, earlier treatment is indicated. This can be done in a single block (forehead, orbital frame, maxilla, zygomas) with distraction, a complex procedure [8].

88.6 Facial Clefts

The facial cleft is a rare facial disorder. Tessier [9] classified the condition considering the orbit as the center with axes radiating from it. Facial clefts occur along these lines. Thus there is considered to be a southern and a northern hemisphere around the orbit with 7 south axes corresponding to 7 north axes, giving a total of 14 axes. Clinically appearances are: hypertelorism, which is considered as a 0-14 cleft; the

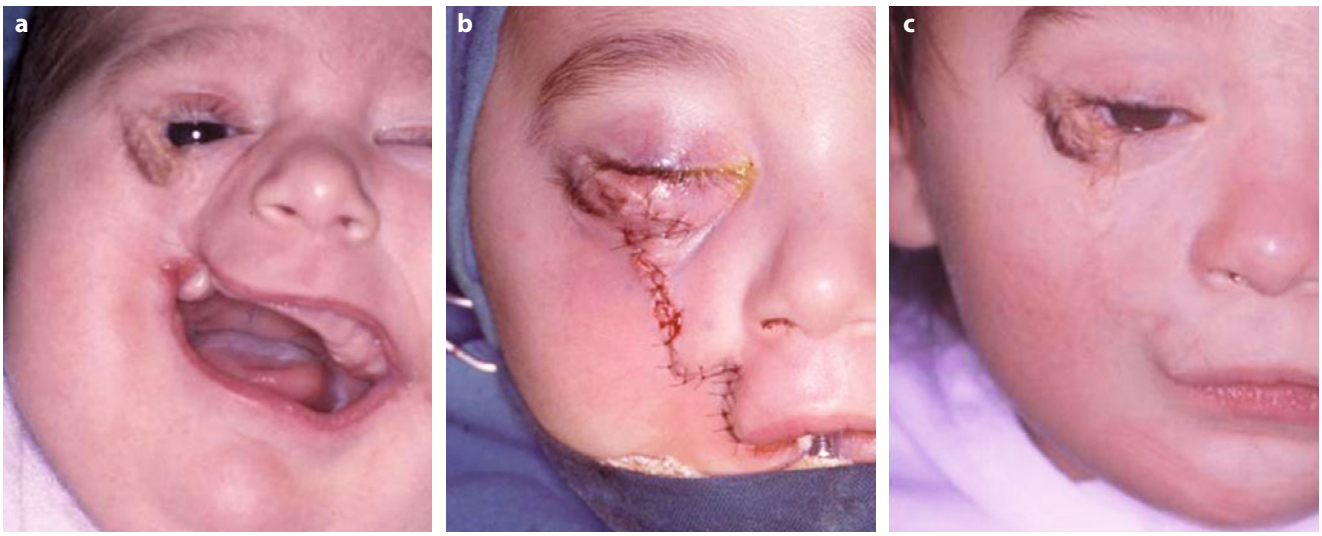

Fig. 88.9 Tessier's n°5 facial cleft treated with local flaps

typical facial cleft is considered as 5; a lateral dermoid cyst on the orbit (cleft 9); and upper or lower, medial or lateral eyelid colobomas.

Hypertelorism, is corrected by osteotomy during early life (4–6 years). This operation removes the bone excess between the orbits that are mobilized by osteotomies and medially transposed and the nasal process is rebuilt by bone graft. If hypertelorism is associated with craniofaciostenosis, it is possible to correct both by so-called facial bipartition to bring the two facial parts and the orbits closer to the midline. The facial clefts are corrected during the first months of life with restoration of bone, muscle and skin continuity of the involved regions, bearing in mind that muscles medially to the cleft may be atrophic or absent (Fig. 88.9).

In eyelid colobomas, restoring corneal protection by reconstructive procedures is necessary to avoid corneal ulceration. Local tissues are used for the reconstruction with good results both from aesthetic and functional perspectives (Fig. 88.10).

88.7 Cysts and Ranulas of the Oral Cavity

A ranula is a mucous cyst (mucocele) of the salivary glands in the floor of the oral cavity. The sublingual gland is most frequently responsible for the formation of ranula. The estimated incidence of congenital ranula is 0.74% with only 5 reported cases diagnosed prenatally. A simple ranula (Fig. 88.11), presenting as a mass in the oral cavity with a translucent azure-blue color, mobile and painless on palpation, can be distinguished from a ranula in the neck, which may threaten airways patency [10]. The most common treatment consists of ranula and sublingual gland excision. Marsupialization only has a significant failure rate.

Mucoceles are the equivalent of ranulas but in different locations, arising from the lower lip (80%) or more rarely the mucosa of cheek, palate or upper lip. They clinically manifest as soft translucent blue or bluish, mobile painless swellings. Treatment is surgical.

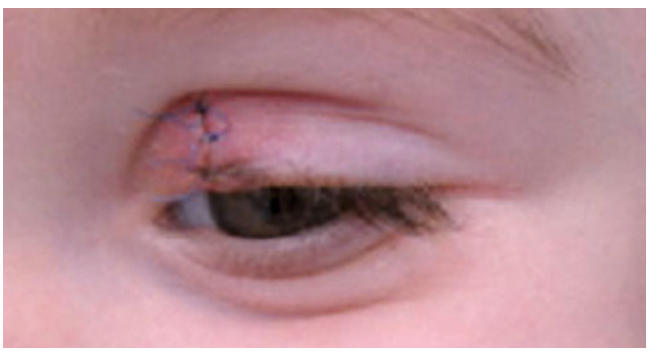

Fig. 88.10 Upper lid coloboma treated with local flaps

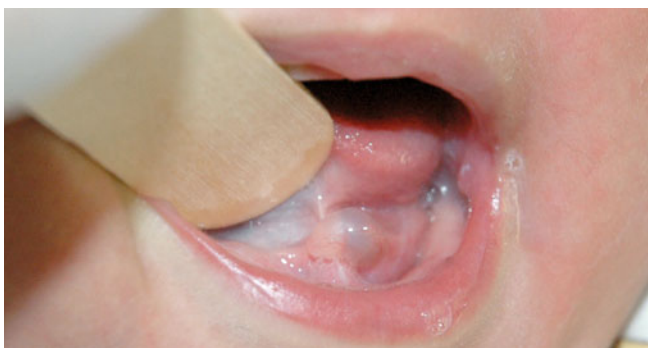

Fig. 88.11 Ranula of the floor of the mouth

88.8 Other Cysts of the Oral Cavity

Other types of congenital cysts may occur at the oral cavity [11], though more rarely. They are divided into epidermoid, dermoid, teratogenic and lympho-epithelial cysts. The distinction is based on histological appearance: epidermoid cysts are surrounded only by simple squamous epithelium; dermoid cysts are from squamous epithelium and skin adnexae such as hair follicles or sebaceous glands; teratogenic cysts consist of stratified squamous and often respiratory epithelium and within the cyst there may be skin adnexae and structures derived from mesoderm, ectoderm and endoderm; lympho-epithelial cysts consist of a stratified squamous epithelium surrounded by clusters of lymphoid tissue. The location of these cysts within the oral cavity is rare. Clinical presentation is similar for each type and is as an elastic mass, which is asymptomatic and presents early. Most often the cyst is located on the floor of the mouth and may be located above or below the mylohyoideus muscle. Treatment is by simple excision using an intraoral approach.

88.9 Tongue Malformations

The most relevant tongue malformations are divided into: ankyloglossia, lingual fissures, macroglossia, microglossia and aglossia.

88.9.1 Ankyloglossia (Tongue Tie)

The ankyloglossia is a common condition affecting approximately 2–5% of newborns [12]. It is caused by a short or fibrous lingual frenum or fusion of the ventral surface of the tongue with the floor of the oral cavity (complete ankyloglossia) (Fig. 88.12). The diagnosis is based on clinical signs by assessing the impossibility of reaching the palate with the tongue or protruding the tip beyond the lower teeth. Ankyloglossia can present a serious problem for the young patient during the first months of life if the child cannot suck normally.

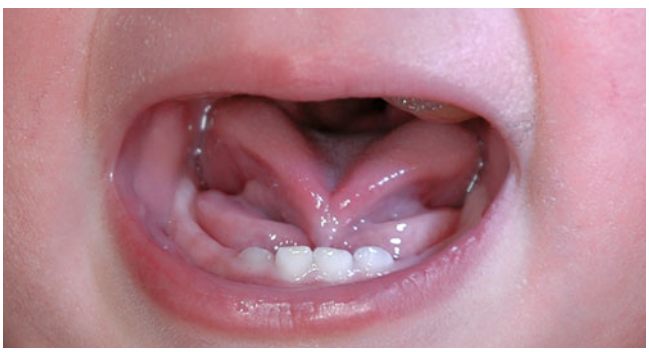

Fig. 88.12 High grade ankyloglossia

Treatment is surgical and involves removal of the frenum, not by a simple cut but by excision that includes the upper bundle of the genioglossus muscle. In the case of fusion of the tongue with the oral floor, grafting may be indicated.

88.9.2 Macroglossia

A congenitally large tongue, or macroglossia, may be due to true hypertrophy of the tongue or occur as an epiphenomenon of a space occupying mass inside the tongue or in the oral floor. True macroglossia may occur with Beckwith-Wiedemann syndrome and Down syndrome [13, 14]. However, true isolated congenital macroglossia is rare condition with a range of clinical manifestations from dental malpositions at the least serious end of the spectrum to excessive mandibular growth and open bite causing speech difficulties. A particularly large macroglossia can cause respiratory disorders. Treatment consists of surgical reduction with a wedge excision at the tip to decrease the length and with a central excision to reduce the width (Fig. 88.13).

88.9.3 Tongue Fissures

Relatively frequent (0.5–5%) and sometimes genetic, they have no clinical significance apart from Melkersson-Rosenthal syndrome where the most important problem is facial nerve impairment.

88.9.4 Microglossia and Aglossia

Lingual agenesis is very rare and related to particular syndromes (e.g., Hanart syndrome). The main clinical effects of microglossia are dental crowding, impaired swallowing and phonation. Therapy consists of ensuring adequate nutritional support. Surgical treatment is difficult and grafting can only be done in less severe forms with functional improvement.

88.10 Congenital Muscular Tortocollis

Congenital muscular torticollis is relatively common with an estimated incidence of 0.3–2%. The etiology is uncertain and there are many etiological theories related to a possible vascular or postural cause. The clinical presentation is with flexion of the neck and head to the affected side with contralateral rotation of the face. There are three subgroups of myogenic

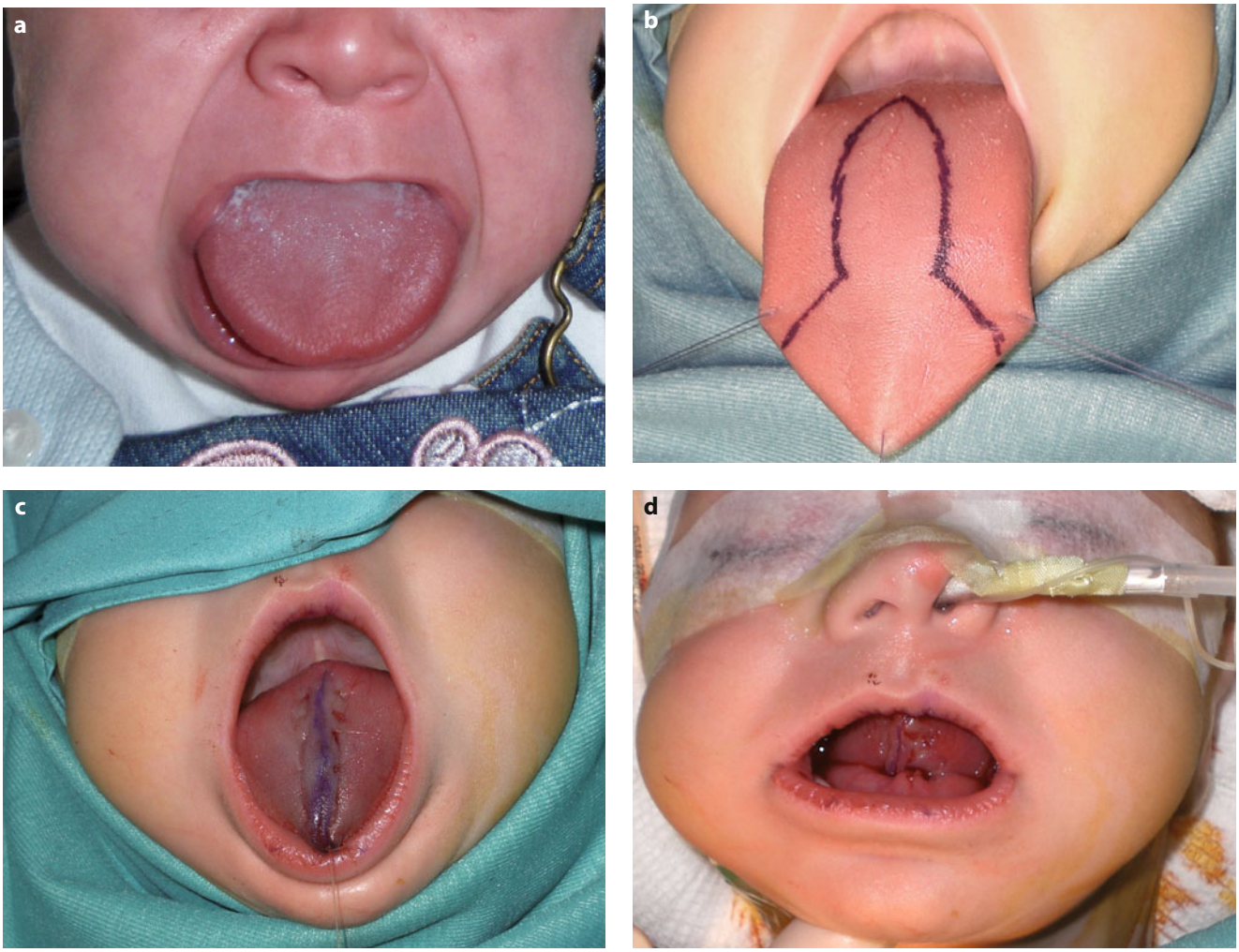

Fig. 88.13 Macroglossia in Beckwith-Wiedemann syndrome treated by resection and remodeling

torticollis: group 1, where a mass is palpable in the sternocleidomastoid muscle; group 2, where there is muscle rigidity and group 3, where the torticollis is not associated with any other symptoms. An association between congenital myogenic torticollis and congenital hip dysplasia has been described [15]. Newborns with hip dysplasia have a doubled risk of developing congenital torticollis compared with the general population. Congenital torticollis should be differentiated from other serious diseases with similar clinical features. Involvement by neurologists, ophthalmologists and otolaryngologists is recommended because 18% of torticollis cases are not myogenic and are caused by brain or spinal tumors, nervous system malformations etc.

After excluding non-myogenic torticollis, therapy for early myogenic torticollis should be started within 4–6 months of age [16, 17]. Conventional treatment consists of frequent physiotherapy sessions of passive stretching. The therapy needs active cooperation of relatives who need to perform traction exercises at home. Botulinum toxin has been used to facilitate stretching if physical therapy alone is not successful. Ninety per cent of cases improve with conservative treatment. The rare non-responders may require surgically correction to prevent secondary craniofacial deformities.

88.11 Ankylosis of Temporo-Mandibular Joint

The temporomandibular joint (TMJ) connects the mandibular condyle to the base of the skull with the interposition of a fibro-cartilaginous disc. It is a complex diarticular joint, meaning that it has two components, allowing both rotation and translation (sliding). Normal anatomy and hence function of the TMJ is essential for mouth opening.

TMJ ankylosis is present when the two articular tips do not move normally or do not move at all. False ankylosis occurs when normal joint mobility is impeded by extra-articular problems, e.g., when there is coronoid process hypertrophy

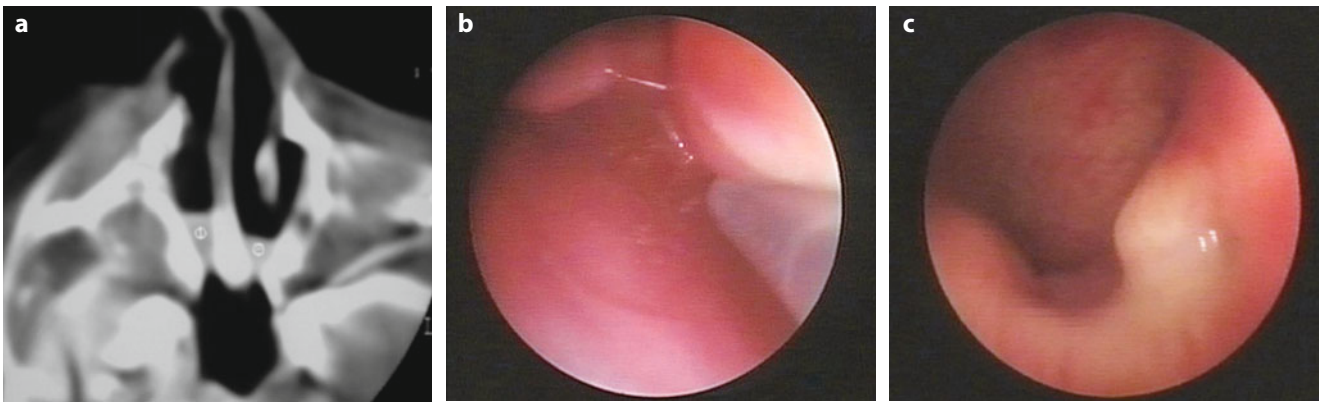

Fig. 88.14 Choanal atresia before and after endoscopic treatment (Case treated by Prof. G. Felisati)

or temporalis muscle fibrosis [18]. True ankylosis is when there is fusion and immobility of the TMJ. Classification is based on the nature of the tissue involved (bone, fibrous or fibro-osseous) and on severity (complete, incomplete) [19].

True congenital ankylosis is extremely rare with an unknown etiology [20]. Few cases have been reported, generally due to the expression of embryogenic errors in association with other malformations. Congenital TMJ ankylosis is more often the consequence of joint damage, which may be infective or iatrogenic (by forceps). The diagnosis is often not made immediately because TMJ has some mobility at birth. True congenital unilateral ankylosis is characterized by an inability to open the mouth associated with facial deformity and chin deviation toward the affected side. In bilateral forms there is symmetrical mandibular hypoplasia with a so-called bird's face. Diagnostic suspicion is confirmed by CT and MR imaging, which can assess the type of ankylosis (fibrous or bony), joint anatomy and mandibular deformity.

Treatment is surgical with resection of the ankylotic bone block and reconstruction of the growth center using a rib bone-cartilaginous transplant. However, early intervention before 5–6 years is characterized by a high relapse rate because of the need for patient cooperation to mobilize the joint.

88.12 Choanal Atresia

Congenital choanal atresia is an anatomic malformation that affects about 1 in 8000–10,000 newborns. It affects girls twice as often as boys. It is more often unilateral than bilateral. In approximately 20–50% of cases, there are other associated malformations.

The etiology is uncertain. Suggestions include a failure of the oro-nasal membrane to perforate, persistence of the oropharyngeal membrane, impaired migration of neural crest or mesoderm cells, or overgrowth of the vertical and horizontal processes of the palatine bone. Atresia consists of a bony-membranous fusion in about 70% of cases and of a complete bony fusion in about 30% of cases.

Congenital choanal atresia, especially when bilateral, may constitute a clinical emergency because newborns are obligate nasal breathers. The presentation is variable, ranging from respiratory embarrassment during feeding to complete postnatal asphyxia with cyanosis. The diagnosis should be suspected in cases where extubation of the infant is impossible. Treatment is to maintain ventilation by oro-tracheal intubation. Correction of the atresia is performed during the first weeks, avoiding the risks associated with tracheotomy.

Several methods have been described for the surgical correction of atresia [21, 22]. The approach may be transnasal

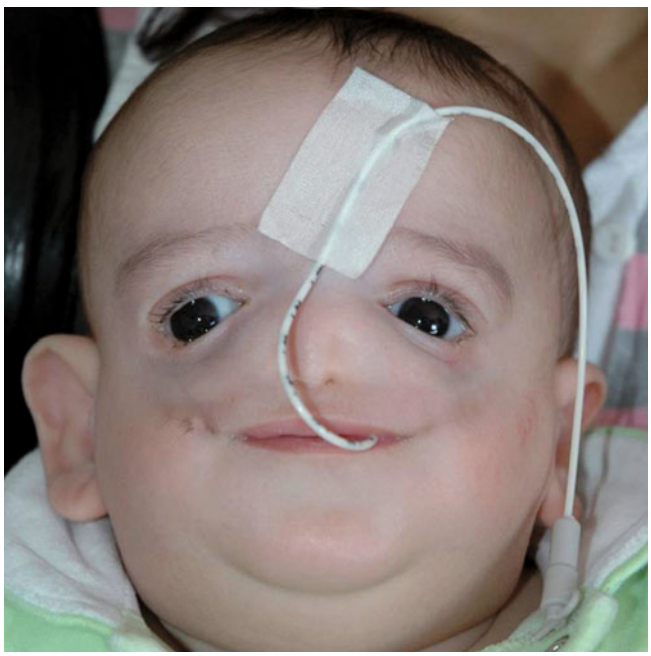

Fig. 88.15 Total arhinia and Franceschetti syndrome

or transpalatine. The latter has a higher complication rate, particularly related to maxillary growth and is therefore reserved for failures of transnasal corrections. A transnasal endoscopic technique provides for perforation with or without resection of the imperforate region and the placement of stents, which need to be in place for at least three months to prevent relapse (Fig. 88.14). Exceptionally a complete arhinia (no external and internal nose) can be observed (Fig. 88.15) and a staged reconstruction can be performed at 5-6 years of age or later.

88.13 Congenital Facial Palsy

Moebius first described congenital facial paralysis in 1888. Congenital facial paralysis is rare. Moebius syndrome is characterized by the presence of paralysis of the V, VI, VII and XII cranial nerves as well as malformations of the eyelids, lacrimal apparatus, palate, and various bony structures, mental retardation, epilepsy and anosmia. Today the term Moebius syndrome is used to describe cases with congenital facial and abducens nerve palsy associated with skeletal malformations. The most frequent causes of congenital facial paralysis are: Bell's palsy (acute dysfunction of cranial nerve VII [the facial nerve] of unknown cause), intrauterine infections and intrapartum trauma. Rarely the cause is an isolated error of embryogenesis.

Clinically, facial paresis may only be diagnosed several months after birth [23]. When bilateral, as in Moebius syndrome, the lack of facial movements is complete. Facial paralysis presents functional problems, the most important of which is corneal exposure due to a failure of eyelid occlusion. This can result in keratitis and corneal ulceration. Associated malformations should be sought.

Treatment of congenital facial paralysis depends on the cause [24–26]. A large proportion of newborns with congenital facial paralysis due to infection or trauma recover facial function spontaneously. Good results have been reported for surgery for facial paralysis that does not resolve spontaneously. The following techniques have been used: muscle rotation to allow movement of the eyelids (temporal muscle), and muscle (gracilis, latissimus dorsi) transfer using microsurgery and reinnervation with a nerve graft from the unaffected side, or, if bilateral paralysis, from the adjacent motor nerves (masseter nerve, a branch of the trigeminal nerve).

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89

Esophageal Atresia

Alfredo Garzi and Mario Messina

Esophageal atresia includes a group of congenital anomalies characterized as interruption of the continuity of the esophagus combined with or without a persistent communication with the trachea [1]. These congenital malformations occur in 1:2500–3000 live births. The overwhelming majority of cases of esophageal atresia are sporadic/non-syndromic, although a small number within this non-familial group are associated with chromosomal abnormalities [1]. Familial/syndromic cases of esophageal atresia are extremely rare, representing less than 1% of the total. Esophageal atresia is 2–3 times more common in twins [2].

The etiology of esophageal atresia is likely multifactorial and remains unknown [3, 4].

89.1 Embryology

The mechanism that causes tracheoesophageal malformations are still unclear, however, the development of reproducible animal models of these anomalies has allowed detailed analysis of the various stages of faulty organogenesis [4]. By contrasting these stages with normal development, it has been possible to identify key developmental processes that may be disturbed during embryogenesis [5].

It is generally accepted that the respiratory primordium appears as a ventral evagination on the floor of the postpharyngeal foregut at the beginning of the fourth week of gestation and that the primitive lung buds are located at the caudal end of this evagination. During a period of rapid growth, the ventrally placed trachea becomes separated from the dorsally placed esophagus [5, 6].

The presence of associated malformations could provide clues as to the possible etiology of esophageal atresia [1, 4, 7].

Such malformations are present in 50% of cases and can occur in distinct patterns. These are non-random associations rather than syndromes because the presence of anomalies in one system makes it more likely that defects exist in another. One of the best-described associations is the VACTERL association, which comprises vertebral, anorectal, cardiac, tracheo-esophageal, renal and limb abnormalities. The pattern of these associations is likely to be dictated by the timing of a possible insult that affects multiple morphogenetic events [1, 7, 8].

89.1.1 Classification

In 1976 Kluth [9] published an extensive *Atlas of Esophageal Atresia* which comprised 10 major types, each with numerous subtypes which is based on the original Vogt classification. It would appear to be more valuable to describe the anatomical anomaly rather than assign a label, which may not be widely recognizable (Fig. 89.1) [10, 11].

89.1.1.1 Esophageal Atresia with Distal Tracheoesophageal Fistula (86%, Vogt IIIb, Gross C)

This is the most common variety in which the proximal esophagus, which is dilated, and the muscular wall thickened ends blindly in the superior mediastinum at about the level of the third or fourth thoracic vertebra. The distal esophagus, which is thinner and narrower, enters the posterior wall of the trachea at the carina or more commonly one to two centimeters more proximally in the trachea. The distance between the blind proximal esophagus and the distal tracheoesophageal fistula varies from overlapping segments to a wide-gap. Very rarely the distal fistula may be occluded or obliterated leading to the misdiagnosis preoperatively of an isolated atresia [10, 11].

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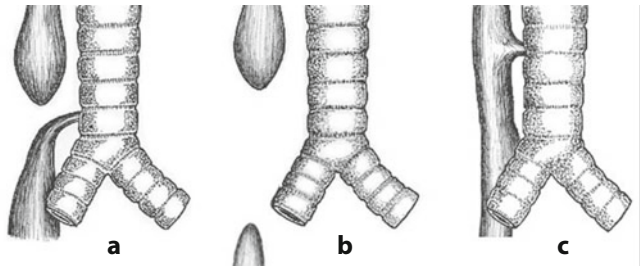

Fig. 89.1 Common anatomical types of esophageal atresia. **a** Esophageal atresia with distal tracheoesophageal fistula (86%). **b** Isolated esophageal atresia without tracheoesophageal fistula (7%). **c** H-type tracheoesophageal fistula (4%)

89.1.1.2 Isolated Esophageal Atresia without Fistula (7%, Vogt II, Gross A)

The proximal and distal esophagus end blindly without any connection to the trachea. The proximal esophageal segment is dilated and thick-walled and usually ends higher in the posterior mediastinum at around the second thoracic vertebra. The distal esophagus is short and ends a variable distance above the diaphragm. The distance between the two ends will determine whether a primary repair is feasible (rarely) or a whether a delayed primary anastomosis or an esophageal replacement should be performed. It is important to exclude a proximal tracheoesophageal fistula in these cases [10, 11].

89.1.1.3 Tracheoesophageal Fistula without Atresia (4%, Gross E)

There is a fistulous connection between an anatomically intact esophagus and trachea. The fistulous tract may be very narrow or 3–5 mm in diameter and is commonly located in the lower cervical region. They are usually single but two and even three fistulas have been described [10, 11].

89.1.1.4 Esophageal Atresia with Proximal Tracheoesophageal Fistula (2%, Vogt III and Gross B)

This rare anomaly needs to be distinguished from the isolated variety. The fistula is not at the distal end of the upper pouch but is sited 1–2 cm above the end on the anterior wall of the esophagus [10, 11].

89.1.1.5 Esophageal Atresia with Proximal and Distal Tracheoesophageal Fistula (<1%, Vogt IIIa, Gross D)

In many of these infants the anomaly was misdiagnosed and managed as proximal atresia and distal fistula. As a result of recurrent respiratory infections, investigations carried out revealed a tracheoesophageal fistula, previously mistaken for a recurrent fistula. With the increasing use of preoperative endoscopy (bronchoscopy and/or esophagoscopy) early recognition of the double fistula is made and total repair performed at the initial procedure.

If the proximal fistula is not identified preoperatively, the diagnosis should be suspected by a large gas leak emanating from the upper pouch during the fashioning of the anastomosis [10, 11].

89.2 Pathophysiology

The motility of the esophagus is always affected in esophageal atresia. The disordered peristalsis more commonly involves the distal esophageal segment. Whether the motility disorder is primarily due to abnormal innervation as evidenced by an abnormality in neuropeptide distribution or secondary to vagal nerve damage occurring during the surgical repair remains uncertain. The resting pressure in the whole esophagus is significantly higher than in normal patients and the closing pressure of the lower esophageal sphincter is reduced. The trachea is also abnormal in esophageal atresia. The abnormality consists of an absolute deficiency of tracheal cartilage and an increase in the length of the transverse muscle in the posterior tracheal wall. When severe, these abnormalities result in tracheomalacia with collapse of the trachea over a 1–2 cm segment in the vicinity of the fistula [12, 13].

89.3 Associated Anomalies

Over 50% of infants with esophageal atresia have one or more additional anomalies. The systems affected are as follows:

- Cardiovascular 29%
- Anorectal anomalies 14%
- Genitourinary 14%
- Gastrointestinal 13%
- Vertebral/skeletal 10%
- Respiratory 6%
- Genetic 4%
- Other 11%.

There is an increased incidence of associated anomalies in pure atresia (65%) and a lower incidence in H-type fistula (10%).

The VATER association first described by Quan and Smith in 1973 [14] consists of a combination of anomalies including vertebral, anorectal, tracheoesophageal and renal or radial abnormalities. This association was later expanded as the VACTERL association to include cardiac and limb defects [1, 7].

Other associations, which may include esophageal atresia are the CHARGE association (coloboma, heart defects, atresia

choanal, retarded growth and development, genital hypoplasia and ear deformities), Potter's syndrome (renal agenesis, pulmonary hypoplasia, typical dysmorphic facies) and Schisis association (omphalocoele, cleft lip and/or palate, genital hypoplasia). Genetic defects associated with esophageal atresia include Trisomy 21 and 18, and 13q deletion. Of the cardiac anomalies, the most common are ventricular septal defect and tetralogy of Fallot. Major cardiac malformations are one of the main causes of mortality in infants with esophageal atresia [1, 7, 15].

The vertebralanomalies in esophageal atresia are mainly confined to the thoracic region and are responsible for later development of scoliosis. The claim that the presence of 13 ribs is associated with long-gap atresia has not been substantiated. Of the gastrointestinal anomalies, the most frequently encountered are duodenal atresia and malrotation, while there is an increased incidence of pyloric stenosis. Miscellaneous anomalies include cleft lip and palate, omphalocoele, lung abnormalities, choanal atresia and hypospadias [15, 16].

89.4 Genetic Counseling

The majority of cases of esophageal atresia are sporadic/non-syndromic, although a small number within this non-familial group are associated with chromosomal abnormalities. Familial/syndromic cases of esophageal atresia are extremely rare. Esophageal atresia is 2–3 times more common in twins. The overall risk of esophageal atresia recurrence in a sibling of an affected child is about 1% [6, 7].

89.5 Clinical Description and Diagnosis

The diagnosis of esophageal atresia may be suspected prenatally by the finding of a small or absent fetal stomach bubble on ultrasound scan performed after the 18th week of gestation. Overall the sensitivity of ultrasonography is 42% but in combination with polyhydraminos the positive predictive value is 56% [17, 18].

Available methods of improving the prenatal diagnostic rate include ultrasound examination of the fetal neck to view the blind-ending upper pouch and to observe fetal swallowing and magnetic resonance imaging [19–21].

The newborn infant of a mother with polyhydramnios should always have a nasogastric tube passed soon after delivery to exclude esophageal atresia. Infants with esophageal atresia are unable to swallow saliva and are noted to have excessive salivation requiring repeated suctioning. A plain X-ray of the chest and abdomen will show the tip of the catheter arrested in the superior mediastinum (T 2–4) while gas in the stomach and intestine signifies the presence of a distal tracheoesophageal fistula (Fig. 89.2). The absence of gastrointestinal gas is indicative of an isolated atresia. A fine bore catheter may curl up in the upper pouch giving the false impression of an intact esophagus or rarely it may pass through the trachea and proceed distally into the esophagus through the fistula [17, 19].

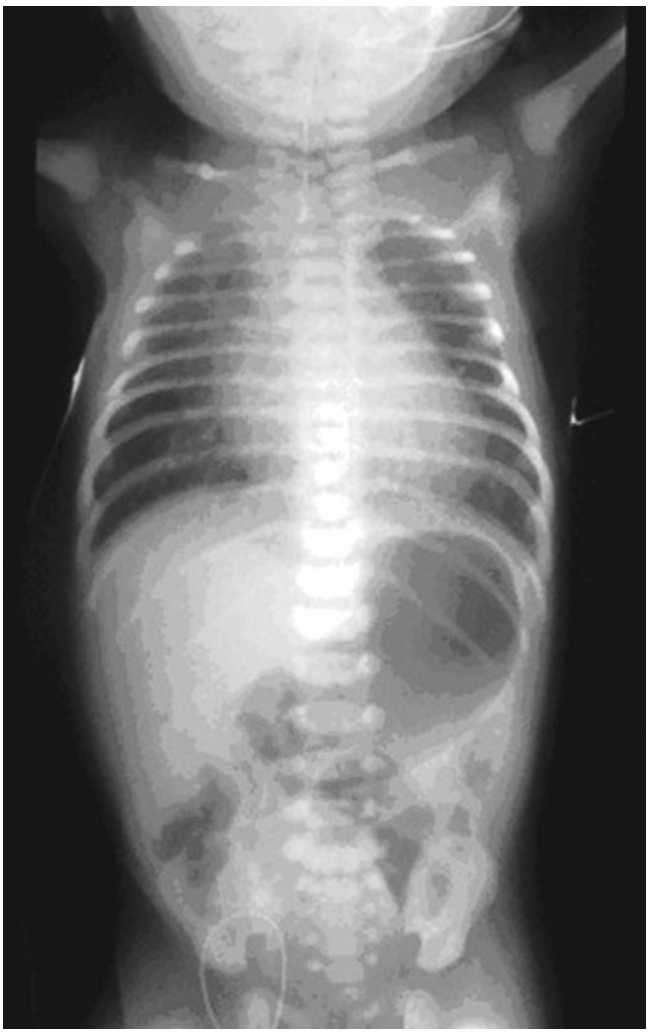

Fig. 89.2 Plain X-ray of the chest and abdomen showing the radioopaque tube in the blind upper esophageal pouch. Air in the stomach indicates the presence of a distal tracheoesophageal fistula

89.6 Management

89.6.1 Preoperative

Once the diagnosis of esophageal atresia has been established, the infant will need to be transferred to a pediatric surgical centre. A suction catheter, preferably of the double lumen type, is placed in the upper esophageal pouch to suction secretions and prevent aspiration. The infant is placed on its side in the

portable incubator while monitoring the usual vital signs. Vascular access should be provided as a precautionary measure but intravenous fluid administration is not usually necessary [16].

The preterm infant with respiratory distress requires special attention. Clearly there is a need for endotracheal intubation and mechanical ventilation. In addition, there is the added risk of gastric over-distension and rupture of the stomach due to escape of respiratory gases down through the distal fistula into the stomach due to the increased pulmonary resistance. This sequence of events can be minimized by positioning the end of the endotracheal tube distal to entry of the tracheoesophageal fistula and by applying gentle low pressure ventilation [13, 16, 17].

All infants with esophageal atresia should have an echocardiogram prior to surgery. The ECHO and/or MR will define any structural anomaly of the heart or great blood vessels and occasionally may indicate a right-sided aortic arch, which occurs in 2.5% of cases. These diagnostic tests determine the side of approach for the operative repair [15].

89.6.2 Risk Categorization and Prognosis (Outcome)

In 1962, Waterston proposed a classification of infants born with esophageal atresia into three groups "with different chances of survival". The classification based on birth weight, associated anomalies and pneumonia comprised:

- Group A Over 5 ½ lb (2500 g) birth weight and well.
- Group B
  1. Birth weight 4–5 ½ lb (1800–2500 g) and well
  2. Higher birthweight, moderate pneumonia and congenital anomaly
- Group C
  1. Birth weight under 4 lb (1800 g).
  2. Higher birth weight and severe pneumonia and severe congenital anomaly.

Over the last 40 years there has been a steady improvement in the overall survival rate due to early diagnosis and prompt referral, improvements in preoperative care and diagnosis and treatment of associated anomalies, advances in anesthetic techniques and sophisticated neonatal intensive care [11].

The Spitz classification for survival in esophageal atresia is:

- Group I Birth weight over 1500 g with no major cardiac anomaly.
- Group II Birth weight less than 1500 g or major cardiac anomaly.
- Group III Birth weight less than 1500 g PLUS major cardiac anomaly.

Major cardiac anomaly was defined as either cyanotic congenital heart disease that required palliative or corrective surgery or non-cyanotic heart anomaly that required medical or surgical treatment for cardiac failure [12].

89.6.3 Selection for Non-Treatment

Infants with Potter's syndrome (bilateral renal agenesis) and trisomy 18 which is fatal in the first year of life in over 90% of affected infants should be offered the option of no active treatment. Similarly, infants with totally uncorrectable major cardiac defects or with Grade IV intraventricular hemorrhage should be considered for non-operative management [11, 12].

89.6.4 Emergency Ligation of the Distal Tracheoesophageal Fistula

Generally, the operative correction of an esophageal atresia is not regarded as an emergency procedure. The one exception is the preterm infant with severe respiratory distress syndrome requiring ventilatory support. Ventilatory gases escaping down the distal fistula result in gastric distension, which further impedes respiratory function. With progressively increasing gastric distension, the stomach may eventually rupture causing a tension pneumoperitoneum, which renders ventilatory support even more difficult [12, 16, 17].

Many maneuvers have been advocated to alleviate the problem including positioning of the endotracheal tube distal to the fistula. However, if the fistula is sited at the level of the carina, this maneuver is impossible to achieve. Others have advocated blocking of the fistula by a Fogarty catheter passed at bronchoscopy. The affected infants are usually preterm and in critical respiratory status. The smallest caliber bronchoscope will not permit ventilation while maneuvering a Fogarty catheter into the distal esophagus in a cyanotic infant will aggravate the condition and further exacerbate the hypoxia [21, 22].

89.6.5 Operative Approach

The operation is performed under general endotracheal anesthesia with dependable vascular access and employing gentle ventilatory pressure so as not to produce gastric distension (Fig. 89.3) [22, 23].

Intraoperative endoscopy Preliminary bronchoscopy may be carried out to define the site of entry of the distal tracheoesophageal fistula and to assess the presence of tracheomalacia. An alternative is to carry out an esophagoscopy to define the length of the upper esophagus and to exclude an upper pouch fistula, which is more common with isolated esophageal atresia [24–26].

Position The infant is placed on the left side with the right arm across the front of the chest for a right posterolateral thoracotomy.

Incision A curved incision centred 1 cm below the inferior angle of the scapula approximately 5–6 cm long is made. The muscle of the chest wall may either be split or divided with

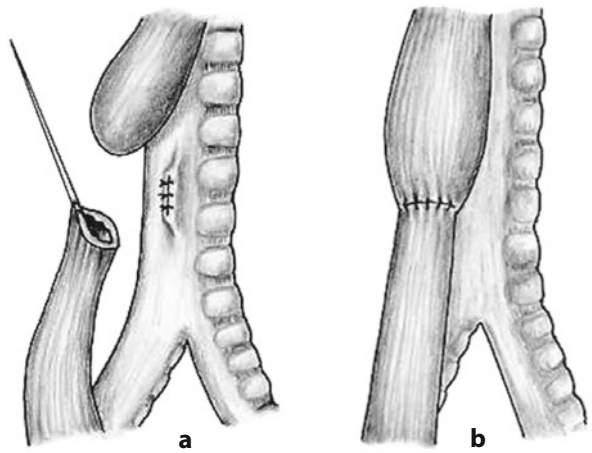

Fig. 89.3 The operative repair of an esophageal atresia and distal tracheoesophageal fistula. **a** Ligature of the fistula. **b** Anastomosis

electrocautery taking care to preserve the long thoracic nerve to serratus anterior. The thorax is opened through the 4th or 5th intercostal space by dividing the intercostal muscles or by entry through the bed of the unresected rib.

Extrapleural Approach Has the advantage of conferring protection of the pleural space in the event of an anastomotic leak. Commencing posteriorly, the pleura is gently freed off the chest wall using blunt dissection. The dissection proceeds into the mediastinum to provide good access to the esophagus. The extrapleural approach is slightly more time-consuming and has theoretically advantages over the transpleural approach still used by many surgeons [21, 27].

Exposure of the esophageal segments The azygos vein is the first structure encountered on entering the mediastinum. The azygos vein is gently mobilized and divided between ligatures to expose the esophagus. The distal esophagus usually lies directly deep to the azygos vein and is identified by the vagus nerve coursing over its anterior aspect [28–30].

Repair of the anomaly The distal esophagus is mobilized. A marking seromuscular suture is placed in the lateral wall of the distal esophagus to assist with orientation. The distal esophagus is dissected to the level of the fistula and the upper and lower extent of the fistula is marked with fine non-absorbable sutures before dividing the esophagus just distal to the fistula. The tracheal side of the fistula is closed with interrupted 5.0 sutures to achieve an air-tight closure. The tracheal closure may be tested by instilling warm saline over the suture line while the anesthesiologist expands the lungs. An end-to-end anastomosis between the proximal and distal esophagus is fashioned using interrupted full-thickness fine sutures. If there is a wide gap, the distal esophagus can be mobilized safely well down towards the diaphragm. Just prior to the final suture being tied a transanastomotic fine-calibre nasogastric tube may be passed. This allows gastric decompression in the early postoperative course and provides a route for early enteral feeding [22, 24, 31].

Methods to overcome a wide gap Various maneuvers have been proposed to overcome a wide gap but in our experience a very tense anastomosis can be achieved in most cases and if the infant is subsequently electively paralyzed and mechanically ventilated for approximately 5 days postoperatively, the anastomosis will heal without leakage. Others have proposed tubularization of the upper pouch after creating a flap, circular myotomy of the upper pouch or abandoning any attempt at initial primary anastomosis awaiting delayed primary anastomosis 6–12 weeks later [32, 33].

The thoracotomy incision is now closed with or preferably without intercostal drainage especially if the procedure has been totally extrapleural and a technically satisfactory anastomosis has been performed. The procedure can be carried out thoracoscopically but this requires advanced skills in minimal invasive surgery [23, 34, 35].

89.6.6 Postoperative Management

In all other instances, regular pharyngeal suction is necessary for the first few postoperative days. The suction catheter should be clearly marked to prevent the tube being passed to the site of the anastomosis and causing damage. Transanastomotic nasogastric feeds may be commenced on the second or third postoperative day and when the infant is swallowing saliva, oral feeds may be started. It is not regularly perform a follow-up contrast study but if there is any doubt as to the integrity of the anastomosis a water-soluble contrast study is carried out [22].

89.6.7 Complications and Outcomes

89.6.7.1 Anastomotic Leaks

These occur in 15–20% of patients but in only one-third or less is there a major disruption. Major leaks occur in the early postoperative period (<48 hours) and present with life-threatening tension pneumothorax. Minor leaks may be detected on the routine contrast study usually performed on the 5–7th day postoperatively [12, 21].

89.6.7.2 Anastomotic Strictures

These develop in 30–40% of cases most of which will respond to one or two dilatations. Risk factors that have been implicated in stricture formation include anastomotic tension, anastomotic leakage and gastroesophageal reflux. Endoscopic dilatation of the stricture can be carried out either at rigid esophagoscopy using semi-rigid bougies (Savary-Gillard) of progressively larger calibre or by balloon dilatation introduced either at fluoroscopy or during flexible

endoscopy. At the end of the procedure, contrast is introduced into the esophagus to ensure that there has been no perforation and to establish the effectiveness of the dilatation [33]. Only rarely is it necessary to resort to resection of an intractable anastomotic stricture.

89.6.7.3 Recurrent Tracheoesophageal Fistula

The incidence of recurrent tracheoesophageal fistula is between 5–14%. A recurrent fistula should be suspected if the infant manifests respiratory symptoms (coughing during feeds, apneic or cyanotic episodes) or has recurrent respiratory infections after "successful" repair of the esophageal atresia [12, 17, 32]. The diagnosis may be suspected on the plain chest radiograph, which shows an air esophagogram.

Bronchoscopic examination will reveal the recurrent fistula at the site of the original tracheoesophageal fistula. It is essential to pass a fine ureteric catheter across the fistula into the esophagus and to view the catheter in the esophagus at endoscopy [22].

89.6.7.4 Gastroesophageal Reflux (GOR)

GOR is common in all infants following repair of esophageal atresia – significant reflux occurs in around 40% of cases, about half of whom will require surgical management. GOR is more common following anastomosis under tension and after delayed primary repair. Incompetence of the lower esophageal sphincter mechanism may be due to a primary neurogenic disturbance inherent in the development of the esophageal atresia or to technical factors involved in the repair or it may be due to shortening of the intra-abdominal esophagus and/or abolishment of the gastroesophageal angle of His [36–38].

Symptoms of GOR are similar to those of recurrent fistula with acute or chronic respiratory problems but also include recurrent vomiting and stricture formation. The diagnosis can be established on contrast swallow, pH monitoring and endoscopy and biopsy of the distal esophagus. Anti-reflux medication including gastric acid suppression is only successful in about half the cases. Surgical treatment is problematic given the inherent dysmotility present in the distal esophagus. A fundoplication may in itself produce a functional obstruction at the gastroesophageal junction. The failure rate of fundoplication carried out in the first three months of life is excessively high. With regard to the nature of the wrap, there are advocates for partial (Thal) as well as for a short, floppy wrap (Toupet or Nissen type) [35, 36].

89.6.7.5 Tracheomalacia

Tracheomalacia may be defined as a structural and function weakness of the trachea resulting in partial and occasionally complete respiratory obstruction. The structural abnormality comprises a deficiency in the cartilage in the tracheal rings and an increase in the length of the transverse muscle. The result is that the airway collapses during expiration causing expiratory stridor, which varies in severity from a hoarse barking cough, to recurrent respiratory infection to acute life-threatening episodes of cyanosis or apnea. The incidence of tracheomalacia is around 10% and about half will require surgical correction [29].

The definitive treatment consists of aortopexy in which the ascending and arch of the aorta are elevated anteriorly towards the sternum. The result is usually dramatic with immediate resolution of the obstruction to the air passages.

89.6.7.6 Dysmotility

This affects the distal esophagus particularly in relation to abnormal coordination of contractions, which in fact can be seen on contrast studies of the esophagus. The dysmotility is a major factor in the long-term swallowing problems encountered in these children [28].

89.6.7.7 Respiratory Function

During infancy and for the first three years of life, patients with esophageal atresia suffer increased frequency of respiratory infections. The tendency to respiratory infections has variously been attributed to esophageal dysmotility and/or gastroesophageal reflux with recurrent aspiration or to a primary respiratory abnormality [16, 17].

89.6.7.8 Congenital Esophageal Stenosis

Congenital esophageal stenosis due to tracheobronchial remnants in the distal esophagus in an infant with esophageal atresia is a rare but well documented phenomenon. It is thought to arise as a result of defective separation of the trachea from the esophagus. It may be recognized at the time of the initial repair of the esophageal atresia when passage of a catheter into the stomach is impeded. Alternatively, symptoms develop quite early with dysphagia and regurgitation of solid food [33, 35].

89.7 Esophageal Replacement

The need to replace the esophagus in esophageal atresia is extremely rare and should only be considered in very long-gap situations or where repeated attempts at retaining the host esophagus have failed and the infant's survival is at risk. There are basically three methods of esophageal replacement currently being practiced in children – gastric transposition, colonic interposition and jejunal interposition [12, 38, 39].

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90

Gastrointestinal Malformations

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90.1 Anorectal Malformations

Anorectal malformation (ARM) is a generic term referring to a group of congenital anomalies of the anus and the rectum, ranging from simple "anal stenosis" to complex "cloacal persistence syndrome". It includes a wide spectrum of presentations, of which anorectal agenesis is the most frequent. ARMs are challenging both from diagnostic and surgical points of view. Even prompt and appropriate treatment is likely to result in lifelong disability due, for example, to incontinence and constipation.

ARMs affect 1 every 4000 births. "High" and "intermediate" forms (anorectal agenesis) are more common in boys (M:F rate = 3:1), whereas "low" forms are more common in girls (M:F = 1:3) (see classification below) [1].

90.1.1 Embryogenesis

The anus and its controlling sphincter muscle complex originate from an ectodermal introflexion that joins the anorectal canal (the end of the primitive gut) and has endodermal origins. Damage at these stages results in an anorectal malformation (ARM) due to incomplete embryological evolution of the primitive "cloaca". At the 4th week of development, the cloaca divides from the cloacal membrane in two parts. The urethra and rectum combine to form the external cloaca. The allantois, the primitive gut and mesonephric ducts form the internal cloaca, which is later divided by a uro-rectal septum into an anterior "uro-genital sinus" and posterior "anorectal sinus" [1]. The "cloacal membrane" then develops into the ventral genital tubercle, the lateral genital folds and the posterior anal tubercles.

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The reasons for interruption of this developmental process are incompletely understood. They give rise to different forms of anorectal anomalies, from a simple "imperforate anus" to multiple forms of "anorectal agenesis", where the rectum ends as a fistula in the neighbouring organs (bladder or urethra in the male, vagina in the female), and the extremely rare "rectal atresia", where the anal orifice is apparently normal but the anal canal ends blindly after few centimetres, and the rectum is atretic.

The "rectal" group of the ARM arises from defects of the internal cloaca, whereas the "anal" group is due to defects of the external cloaca (perineum, genital folds, and proctodeum).

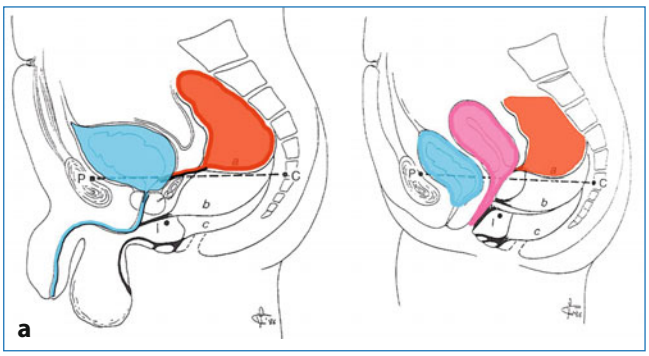
90.1.2 Classification

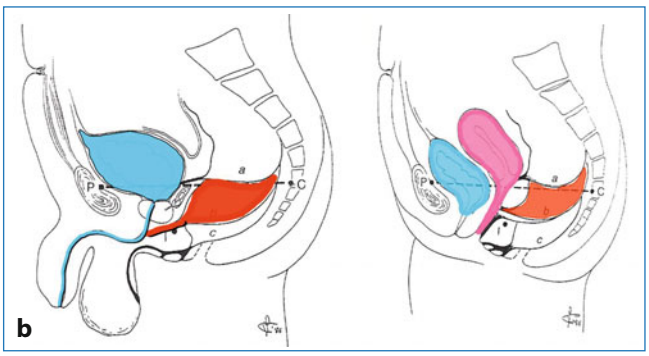
The Melbourne Classification (1970), modified by Wingspread (1984), is used to classify the different forms of ARM. These are based on radiological assessment of the level of the ARM: an abdominal X-ray 24 hours after birth images the position of intestinal gas relative to the rectal pouch by using a radiopaque spot painted in the perineal area to mark where the anal orifice should be [2]. A line is then drawn on a lateral radiograph from the middle of the pubic bone to the sacrococcygeal joint at the level of the "ischiatic point" ("the pubococcygeal [p-c] line"). The classification is based on the level of the rectal pouch in relation to this line.

- "High ARM" is when the intestinal air of the rectal pouch stops above the pubo-coccygeal (p-c) line (Fig. 90.1a).
- "Intermediate ARM" is when the intestinal air stops between the p-c line and the ischiatic point (Fig. 90.1b).
- "Low ARM"is when the intestinal air stops below the p-c line and the ischiatic point, close to the perineal skin (Fig. 90.1c)

In the past these X-rays were taken with the baby held by the legs and the head down (so called "invertography"), but this has been shown to be useless and a simple plain X-ray is sufficient.

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.





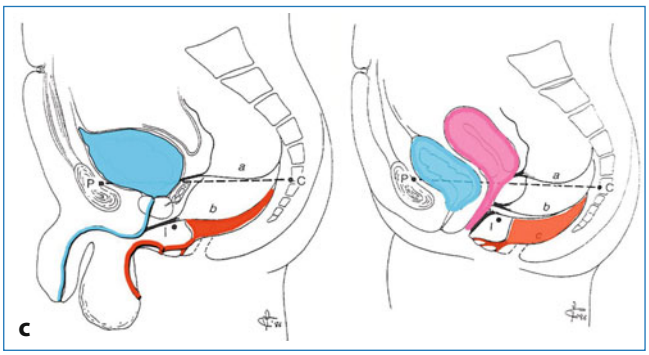


Fig. 90.1 **a** Classification of "high" forms of ARM in males and females. Note: rectal agenesis above the pubo-coccygeal line and its relationship to genital or urinary organs. **b** Classification of "intermediate" forms of ARM. Note: rectal agenesis is below the pubo-coccygeal line and there is a fistula. **c** Classification of "low" forms of ARM. The anal canal is incomplete but very far from the perineal skin. Mostly it presents with a cutaneous fistula

In the Wingspread classification, the cloaca is considered as a separate form ("the clover syndrome"), also subdivided into three forms: high, intermediate and low, according to the length of the common canal into which the outputs of the rectum, urethra and vagina flow. Recently Peña proposed a new classification system. It is purely descriptive with therapeutic and diagnostic implications for males and females. It does not define ARM as "high", "intermediate" or "low" but relies on clinical features and the presence or absence of a fistula [2].

90.1.3 Associated Anomalies

Associated anomalies are frequent, probably due to cloacal subdivision occurring between the 4th and the 6th week, i.e., at the start of organogenesis. The incidence of associated anomalies is 50–60% and varies with the form of ARM. In the "rectal" forms (high and intermediate ARM), the incidence and severity of associated malformations is almost twice that of the "anal" forms (low ARM). In decreasing order of frequency, they involve the uro-genital, skeletal, digestive, cardiac, nervous, pulmonary systems.

Uro-genital system The most frequent anomalies (20–30% with low ARM, 50–70% with high/intermediate ARM). Vesico-ureteral reflux (VUR) is the most frequent anomaly in the female. Obstructive uropathies and neurological bladder are extremely common in association with sacral agenesis.

Skeleton Vertebral anomalies are common (40% with "rectal" ARM, 17% with "anal" ARM). Most are localized to the sacrum or coccyx (sacral agenesis or hemisacrum). Because these anomalies affect almost half of high forms, imaging (radiography, CT and MR) is required to identify a "tethered cord", i.e., caudal traction on the medullar cone causing abnormal neurological signs in the legs or a neuropathic bladder. Neurosurgical referral is indicated if there is a tethered cord.

Intestinal system Incidence is about 10% (15% with "rectal", 7% with "anal" ARMs). Esophageal atresia affects 10%, duodenal atresia 2%, and Hirschsprung's disease 3-5%.

Cardiovascular system Cardiovascular anomalies are present in 9% of cases, regardless of the form of ARM.

Other Other associated anomalies may also occur (cleft lip, hydrocephalus, rachischisis).

VATER association An association of anomalies due to: Vertebral anomalies (V), Anorectal agenesis (A), Tracheo-esophageal fistula (TE) with or without esophageal atresia, Renal dysplasia (R). The association may be more complex (VACTER, VACTERL) if a cardiac defect (C) and/or limb hypoplasia (L) are present.

90.1.4 Symptoms

ARMs usually present immediately after birth at clinical examination of the perineal area. If the diagnosis is missed, the clinical features are those of intestinal obstruction: abdominal distension, a failure to pass meconium, bile-stained vomiting. If there is a perineal fistula, meconium may be passed normally with normal growth during infancy but chronic constipation and sub-acute intestinal obstruction.

90.1.5 Diagnosis

ARM is not usually diagnosed prenatally. Sometimes there is colonic dilatation without polyhydramnios. The condition may be suspected when a skeletal defect is identified (radial or sacral aplasia), particularly in the presence of an associated uropathy or cardiomyopathy. Simple examination of the perineal area after birth is usually sufficient to make the diagnosis. In low forms, meconium is sometimes passed from a cutaneous fistula in the perineum. In males, this orifice can be observed ventrally along the raphe line anywhere from the anus to the tip of the penis. In females, the orifice can be in the anterior perineal region, at the vaginal vestibule or vulva.

In high or intermediate forms, there may be an internal rectal fistula (recto-urethral fistula in males; recto-vaginal fistula in females) and meconium may be passed from the urethra or vagina. In patients with persistence of the cloaca, meconium and urine may be passed from a single orifice, the common channel.

Further examination (e.g., sacral radiography, cardiac ultrasound, urinary ultrasound, CT or MR, cysto-urethrography and urodynamics) should exclude associated abnormalities, such as vesico-ureteral reflux, a neurological bladder or "tethered cord". Vaginoscopy may be useful to distinguish a duplex from a septate vagina and to identify any fistula, which is most common on the right. In cloacal forms, visualisation of the common channel by endoscopy is required to measure its length. When a temporary colostomy has been fashioned, distal colonography is useful to determine the position of the fistula.

90.1.6 Therapy

The management of low forms is different to high or intermediate variants.

In low ARM, complete surgical correction is done within 24–48 hours after birth. The rectum is normally positioned in the "pubo-rectal sling" and a new functioning anal orifice is established in its natural anatomic site by identifying the middle of the external sphincter using a muscle electro-stimulator (Fig. 90.2).

In high or intermediate forms, a temporary colostomy is performed before definitive surgical correction. The most usual surgical technique is the "Posterior Sagittal Ano-Recto-Plasty" described by Peña-De Vries (1982) [3]. The baby is placed prone and a sagittal posterior skin incision is made through the intergluteal line, separating the muscle structures "like a book" until the rectal pouch is reached. The fistula is identified, sutured and divided. The rectal pouch is opened and separated from the urethra (in males) or vagina (in females), then positioned in its appropriate anatomic site inside the "muscle complex", which is identified by the electro-stimulator.

A similar technique is used to correct the cloacal malformation, bringing the three separate orifices out on the skin of the perineum.

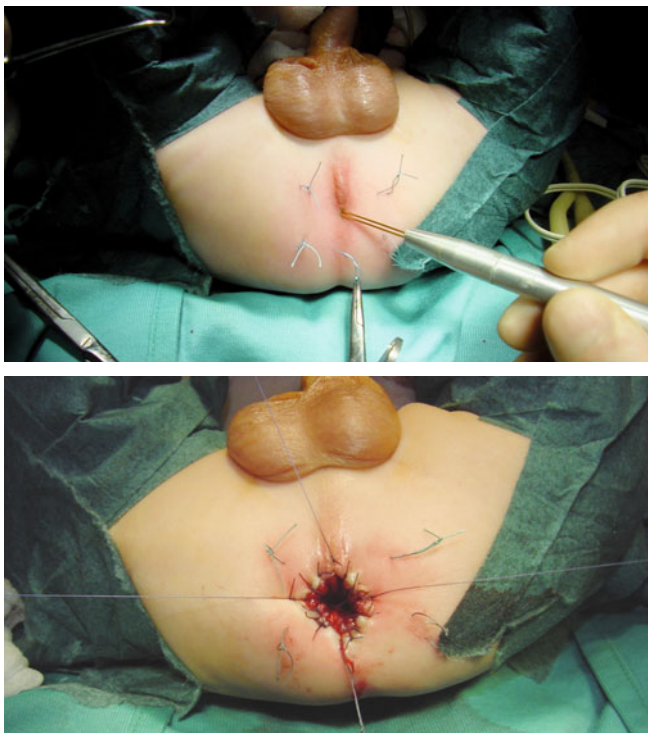


Fig. 90.2 Low ARM. The electrostimulator allows for the identification of the point at which to create the new anus. The rectum has been identified a few millimetres from the perineal skin

Results obtained by Peña's technique are better from a functional (fecal continence) and an esthetic viewpoint than previous surgical techniques that favored an abdomino-perineal approach (Stephens 1953; Rehbein 1965; Swenson 1967; Kiesewetter 1967). The advantages of Peña's technique can be summarized as follows: 1) precise identification of each perineal muscle; 2) optimal surgical visualization; 3) correction at a single operation of each type of ARM (including persistence of the cloaca); 4) optimal siting of the neo-anus (and other orifices in cloacal forms); 5) a good functional result in respect of urinary and fecal continence.

In 1997, Bianchi proposed a technique ("Trans-Ano Proctoplasty TAP") [4] that combined an abdominal with a perineal approach for correcting high and intermediate forms of ARM in males. This technique gained interest because it allowed the malformation to be corrected without splitting the muscle complex. In 2000, Georgeson introduced the "Laparoscopic Assisted Ano-Recto-Plasty" (LAARP), a new mini-invasive technique that developed Bianchi's approach further by using laparoscopy [5]. The fistula is identified, sutured and divided using a three-trocar laparoscopic approach. In Siena, Italy, it is considered the treatment of choice for high forms of ARM, although specific laparoscopic skills are needed.

A video-assisted surgical technique for ARM in females has been proposed by Marcela Bailez [6].

There have been concerns about the lack of data relating to complications and follow-up studies are needed.

90.1.7 Postoperative Treatment

The postoperative care of these children is important. Every child must have repeated postoperative anal dilatations (for 3–6 months) to progressively dilate the muscle complex to achieve an "elastic" anal canal. The colostomy closure should be delayed for at least three months. The patient should be followed for the first 2–3 years during toilet training, and enemas or laxative therapy should be used until there is regular intestinal motility. Continence is graded by a four-point system: 1) clean; 2) stained; 3) sporadic fecal soiling; 4) constant fecal soiling.

90.1.8 Outcomes

Deaths in patients with ARM are confined to those with serious associated life-threatening anomalies. High and intermediate forms are associated with 30–40% disability (e.g., fecal incontinence, neurologic bladder).

90.2 Intestinal Malrotations

This abnormality occurs during the rotation and fixation phases of intestinal development and there is malposition of the ascending colon, which is under the stomach instead of on the right flank. The peritoneum that covers the colon and fixes it to the posterior abdominal wall has to pass in front of the duodenum, and the resulting compression may cause an obstruction. Because the large intestine is mobile, there is also an increased risk of intestinal volvulus. About 1 in 10,000 births show abnormal clinical features, but the incidence at autopsy is 1 in 2–300. These findings underline the fact that anorectal malformations are generally well tolerated and that only 1 in every 500 patients experiences complications. Boys are affected twice as frequently as girls.

90.2.1 Classification

The rotation-fixation process is complex and interruption at any stage results in different forms of malrotation (Table 90.1).

The most frequent forms are [7]:

- Type I A: Lack of rotation also called "mesenterium commune", where the small bowel is in the right hemi-abdomen while the colon is in the left.
- Type IIIA: There is incomplete rotation: the small bowel remains in the right hemi-abdomen and the cecum and ascending colon are in the epigastrium. In this form, parietal adhesions (Ladd's band) between the abdominal wall and colon may cause obstruction by external compression of the 3rd part of the duodenum.

"Volvulus neonatorum" occurs most frequently in types IA and III.

Table 90.1 Types of intestinal malrotation and possible consequences

Type	Anatomy	Clinical aspects
IA	No rotation	Volvulus of the midgut
IIA	No duodenal rotation; normal colonic rotation	Duodenal obstruction; Ladd's bands
IIB	Inverse duodenal rotation; Normal colonic rotation	Intestinal obstruction
IIIA	Normal duodenal rotation; Absent colonic rotation	Duodenal obstruction; Ladd's Bands; Intestinal volvulus
IIIB	Incomplete rotation and fixation	Duodenal obstruction; Ladd's Bands
IIIC	Incomplete fixation of the cecum	Volvulus of the caecum
IIID	Internal Hernias	Obstruction or sub-acute obstruction

90.2.2 Embryogenesis

There are no genetic or familial causes for ARM. The primitive gut completes the anticlockwise rotation between the 4th and the 10th week of gestation and fixes to the posterior abdominal wall when it reaches its definitive position (the ascending colon on the right, the descending colon on the left). Failure of this developmental process constitutes intestinal malrotation [8].

90.2.3 Associated Anomalies

Intestinal malrotation invariably complicates Bochdalek's diaphragmatic hernia, omphalocoele or gastroschisis. Malrotation also occurs with intestinal atresias.

90.2.4 Symptoms and Diagnosis

Antenatal ultrasound does not assist the diagnosis of intestinal malrotation.

Clinical features are those of intestinal obstruction. It may be present as a duodenal obstruction, a volvulus or an internal hernia. Half of infants with ARM show abnormal physical signs during the first weeks of life. The other 50% usually show some evidence of malrotation within the first six months. Later, the presentation is variable [9].

90.2.4.1 Duodenal Obstruction

In neonates and toddlers, the presentation is acute because of "Ladd's bands" that obstruct the third part of the duodenum, giving rise to bile-stained vomiting. Infants and pre-school children may present with cramp-like abdominal pain

90.2.4.2 Intestinal Volvulus

Usually clockwise, constituting a diagnostic and surgical emergency. In about 50% of affected patients, volvolus occurs during the first two weeks of life ("volvulus neonatorum"), with bile-stained vomiting and progressive painful abdominal distension. There may be bloody stool. This is followed by a rapid clinical deterioration and the development of shock, which, if not treated promptly, may be followed by death within hours. In infancy or adolescence, symptoms may be subtle and varied, usually comprising cramp-like pain and vomiting, which may be bile-stained.

Acute appendicitis may be difficult to diagnose when there is a malrotation because, instead of being in the right lower abdominal quadrant, the cecum may be in the left iliac fossa, hypogastrium or elsewhere in the abdomen and acute appendicitis may only be diagnosed at laparotomy or by laparoscopy. There may be malabsorption because of intermittent torsion of the intestinal loops with twisting of lymphatic vessels causing mesenteric lymphoadenomegaly.

May be very difficult but early diagnosis results in a better outcome.

The first investigation for "volvulus neonatorum" is a straight abdominal X-ray [9]. However, this may not be diagnostic because air-fluid levels are generally absent. Volvulus should be suspected when there is no intestinal air and massive abdominal opacification due to a peritoneal transudate. If there is sufficient time, a contrast enema will show the colon on the left side of the abdominal cavity. A contrast medium radiograph of the abdomen may also help. Prompt diagnosis and treatment results in preservation of the intestine, avoiding the serious complication of short bowel syndrome (SBS) because of removal of a large section of necrotic bowel.

90.2.5 Therapy

Surgery is indicated for all types of malrotation.

For types II and III, the operation consists of resection of the Ladd's bands, followed by the identification and treatment of associated volvulus or internal hernia, and repositioning the bowel with the small bowel to the right and the large bowel to the left. A prophylactic appendicectomy should always be performed [10].

In cases of "volvulus neonatorum", surgery should be performed as soon as possible. Following a supraumbilical skin incision, the volvulus is reduced by anti-clockwise rotation of the cyanotic bowel. It is useful to wait about ten minutes to observe return of blood to the gut. Ladd's bands must be carefully resected and the bowel repositioned in the abdomen. If bowel loops appear to be necrotic with no apparent recovery after correction of the torsion, the recommendation is not to resect the intestine immediately but to suture the abdomen with the gut inside, planning for a repeat laparotomy after 24 hours. This conservative approach may allow the necrotic area to become defined allowing a shorter resection. Alternatively, an ileostomy of the healthy bowel may be performed. Recurrence of volvulus is extremely rare.

In the last decade, laparoscopy has also been used to treat symptomatic intestinal malrotation or to identify by imaging patients with possible intestinal malrotation as a group that is prone to volvulus (and therefore requiring treatment) and a group that is not [11]. Laparoscopy can be used to perform an identical procedure to conventional surgery.

90.2.6 Prognosis

Intestinal malrotation uncomplicated by volvulus has a very good prognosis. By contrast, the prognosis for "volvulus neonatorum" is very poor (death and/or SBS in 10–30% of patients). Patients with SBS may survive with lifelong total parenteral nutrition (TPN), but this has a high complication rate (sepsis and liver failure). If there is only 20–30 cm of small bowel but preservation of the ileo-caecal valve a good quality of life is achievable and TPN may be combined with some enteral nutrition. Combined liver and intestinal transplantation carries a poor prognosis.

90.3 Duplications of Alimentary Tract

Intestinal duplications are spherical or tubular structures, which may occur anywhere from esophagus to anus. Tubular duplications may be joined to, and sometimes communicate with, normal bowel.

The incidence is low, representing only 0.1–0.3% of all congenital malformations of the alimentary canal. Table 90.1 shows the regional incidence along the enteric canal. There is no sex prevalence if considered as a single group, but gastric duplications are more likely in girls while boys are more likely to have thoracic duplications.

90.3.1 Embryopathogenesis

Since the morphology varies, it is difficult to establish a single embryological origin [12]. Three different theories have been proposed.

90.3.1.1 Diverticular Theory

In 1905 Kaible observed that in the 4–8 week human embryo there are microdiverticular structures inside the enteric tract. He proposed that persistence and growth of these diverticular structures, which normally disappear, is the basis of the development of duplications. This may explain the origin of spherical but not tubular duplications.

90.3.1.2 Theory of Abnormal Enteric Recanalization

In 1944 Bremer proposed that abnormal recanalization of the intestinal lumen after the solid stage of development of the primitive gut during the 6th–7th week of gestation may result in duplications. However, it has since been shown that the solid stage of human embryo gut development does not usually extend beyond the duodenum.

90.3.1.3 Notochord Split Syndrome

The most satisfactory theory of the origin of gastrointestinal duplications relates to the development of the neuroenteric canal. Saunders (1943) and subsequently Bentley-Smith (1960) established a "split notochord theory", hypothesising that these malformations are dependent on the incorrect separation of the notochordal plate during the presomitic stage. According to these authors, if the notochordal plate fails to migrate as a result of adhesions to the endodermal lining, the spinal canal cannot close ventrally and a tract resembling a diverticulum is established with the primitive gut. This tract may remain open leaving a fistula between the gut and the spinal canal. However in the majority of cases it disappears completely leaving only duplication of the gastrointestinal tract. This theory explains thoracic and caudal duplications, but does not really explain other alimentary duplications and underlines the impossibility of creating a unified model.

90.3.2 Pathology

Duplications are hollow structures with a muscle and a mucosal layer that may contain a small area of heterotopic lining (usually gastric mucosa but sometimes also pancreatic or respiratory mucosa). They often share a common muscular wall and blood supply with the adjacent normal bowel and may communicate with its lumen (more common in the case of tubular duplications which are usually on the antimesenteric border). More frequently there is no communication (cystic duplications are generally on the mesenteric edge).

90.3.2.1 Cystic Duplications

These represent about 90% of cases. Most are located in the small bowel (60%) and do not communicate with the normal intestinal lumen. Must be differentiated from other cystic masses in the abdominal cavity that lack an intestinal mucosal lining.

90.3.2.2 Tubular Duplications

These represent about 10% of cases and are long structures adherent to the normal lumen with which they communicate. Usually located on the antimesenteric border, their length varies from a few centimetres to one meter or more. The duplication may extend the length of the small bowel or the colon. Sometimes abdominal forms may extend to the thorax, causing rare thoraco-abdominal duplications (usually gastroesophageal duplications).

90.3.3 Associated Anomalies

Rare, principally consisting of:

- malformations of the intestinal tract, e.g., esophageal atresia, intestinal atresia;
- vertebral malformations;
- lumbar myelomeningocele or complex genito-urinary malformations in the case of rectal or colonic duplications.

90.3.4 Symptoms and Diagnosis

70% of cases present during the first six months of life. The remaining 30% present during the next eighteen months. Clinical presentation at an older age is exceptional.

When an antenatal ultrasound scan shows an intra-abdominal cyst, cystic duplication should be considered. Tubular duplications are usually not diagnosed antenatally.

The clinical picture depends on the level of the duplication in the alimentary tract, on its morphology (cystic or tubular), and on the mucosal lining. Cystic forms often cause obstruction because of a "mass effect" due to increasing volume because of internal secretions. Sometimes there is volvulus or intussusception with intestinal obstruction.

Tubular forms that are lined with gastric mucosa usually present with intestinal bleeding or perforation.

Clinical features depend on which region of the intestine is affected:

- esophagus: dysphagia, respiratory distress, cardiac disturbances;
- gastrointestinal tract: mass effect, vomiting, obstruction, intestinal bleeding;
- colorectal tract: mass effect, constipation, bleeding.

Diagnosis is by abdominal ultrasound, contrast medium radiography of the abdomen, MRI or CT and scintiscan with 99Tc pertecnectatum to identify tubular forms with gastric mucosa if there is bleeding.

The video-capsule has become a useful diagnostic tool for recognition of the inner orifice of a tubular duplication of the small bowel [13]. Colonoscopy remains the gold-standard for colonic duplications.

90.3.5 Therapy and Prognosis

Cystic duplications must always be excised even if asymptomatic because of the risk of malignancy. For cystic duplications,

intestinal resection with an end-to-end anastomosis is usually performed. For tubular duplications (e.g., esophageal) the mucosal layer may be stripped away. Sometimes colonic duplications may be treated by forming a distal communication between normal and duplicated colon [12]. Rectal forms should be approached by a posterior sagittal incision, for example, Peña's procedure. An alternative approach is by a combined perineal and abdominal approach. A temporary colostomy may be needed. The prognosis is usually good but depends on the form and level of the duplication.

90.4 Persistance of the Omphalo-(mes)enteric Duct

The pathology of the omphalo-enteric duct (incorrectly called "omphalo-mesenteric") consists of a wide range of congenital anomalies all due to missed or partial involution.

90.4.1 Classification

Five different presentations can be recognized, in decreasing order of frequency [14].

- Meckel's Diverticulum.
- Omphalo-enteric fistula, where there is communication between ileum and umbilicus.
- Fibrous cord (obliterated omphalo-mesenteric duct, extending from ileum to umbilicus).
- Umbilical sinus or polyp (remnant of intestinal mucosa at the umbilicus).
- Cyst of the vitelline duct (as a fibrous cord).

90.4.2 Meckel's Diverticulum (MD)

MD is the most frequent, with an incidence of about 82–96%, fibrous cord about 10% and omphalo-enteric fistula between 2 and 6%. Described in 1809 by J.F.Meckel, a German anatomist who defined its embryological origin from the vitelline duct [14]. The MD is a "true" ileal diverticulum. Its wall contains all three layers of the intestinal wall, in communication with the ileal lumen and about 20–60 cm from the ileocaecal valve (Fig. 90.3). It is located on the antimesenteric border of terminal ileum. In 75% of cases MD is not connected to the internal abdominal wall; in the remaining 25% its tip is joined to the umbilicus. Usually MD has its own vascular pedicle deriving from the vitelline arteries. In 25% of cases it is lined with heterotopic tissues (islets of gastric mucosa or, more rarely, pancreatic parenchyma). MD with heterotopic tissues is three times more likely to cause symptoms than the other forms of MD.

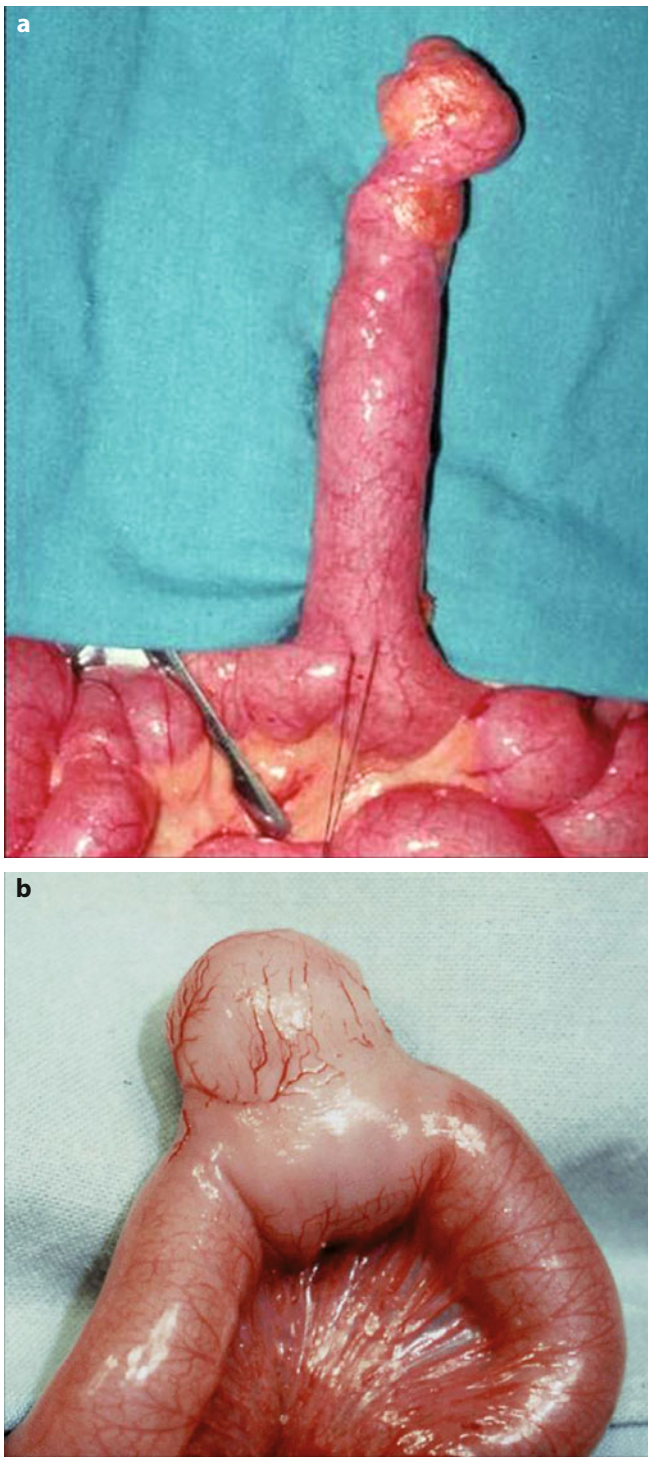


Fig. 90.3 **a**, **b** Meckel's diverticula, please note the wide spectrum of presentation

In a postmortem study, MD was identified in 2% of people. The male:female ratio is 3:1 in symptomatic cases, but was 1:1 when identified at autopsy. Eighty percent of complicated MD present below 10 years of age, 60% younger than 2 years.

90.4.2.1 Embryopathogenesis

MD is the embryonal remnant of the omphalo-mesenteric duct (or vitelline duct), which connects the primitive gut with the yolk sac. It is a consequence of its persistence because of incomplete obliteration, which normally occurs during the 5th–7th week of gestational age [15].

90.4.2.2 Associated Anomalies

MD is frequently associated with other intestinal malformations: omphalocoele (24%), anorectal malformations (11.4%), duodenal or jejunal atresia (12.7%) and esophageal atresia (5%). MD must be looked for at abdominal surgical exploration (either by laparoscopy or laparotomy).

90.4.2.3 Symptoms

Only 4–6% of people with MD will develop symptoms. The incidence of clinical presentation is inversely proportional to age and is most common in males. Complications can be subdivided into five groups:

- intestinal bleeding (31–35%)
- intestinal obstruction (27–30%)
- diverticulitis (22–25%)
- enteric fistula or omphalitis (4–10%)
- other (1–10%).

Usually symptoms manifest within 2 years after birth, particularly with intestinal obstruction or bleeding (in older patients, the main presentations are bleeding or diverticulitis).

Intestinal obstruction may be due to intussusception of the MD or volvulus. Intussusception causes recurrent abdominal pain, vomiting, rectal bleeding and a palpable lower quadrant abdominal mass. Volvulus may occur because intestinal loops twist around the fibrous remnant of the vitelline duct, which is joined to the abdominal wall, causing intestinal ischemia and necrosis.

Bleeding is a consequence of peptic ulceration due to hydrochloric acid secreted by the islets of heterotopic gastric mucosa lining the inner mucosa of the MD. Peak incidence is during the first 2–3 years of life.

90.4.2.4 Diagnosis

When intestinal bleeding is seen, MD should be considered. An abdominal scintiscan with Tc-99 pertecnectatum (Fig. 90.4) [16] is used to confirm the presence of heterotopic gastric mucosa. Introduced by Jewett in 1970, it is based on the preferential uptake of the radioisotope by gastric mucosa. Sensitivity can be increased to about 80% by the administration of pentagastrin or cimetidine. When bleeding stops, the diagnosis can be also be made by video-capsule to identify the orifice of MD [17]. If the diagnosis remains uncertain, videolaparoscopy has been used.

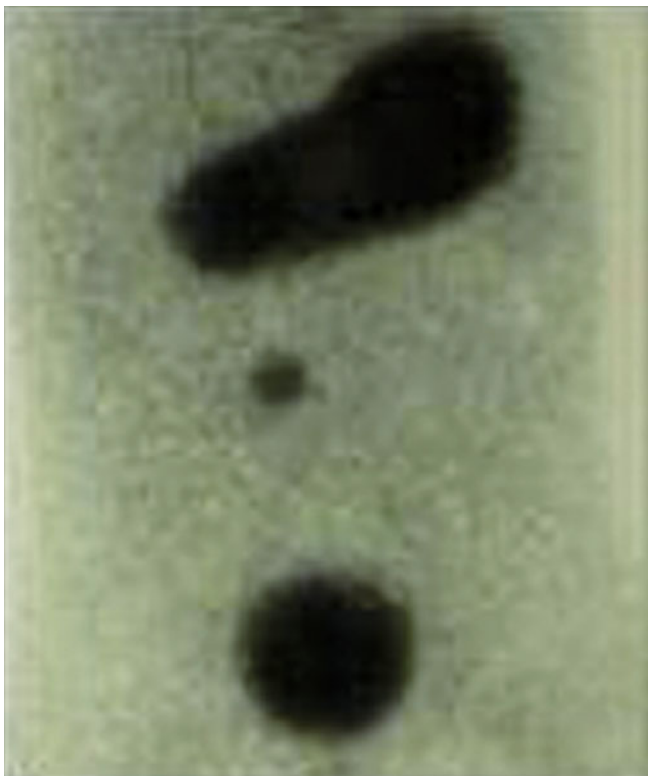


Fig. 90.4 Scintiscan with Tc99. At the top you see the gastric accumulation of radionuclide; at the bottom there is the bladder filled with radioactive urine and among them there is a little accumulation in correspondence of an isle of gastric mucosa inside the MD

90.4.2.5 Therapy and Prognosis

Treatment is by resection of the MD or of the intestinal loop that contains the MD and subsequent ileal anastomosis, either at laparotomy or by laparoscopy [17]. When a MD is found by chance during laparotomy or laparoscopy for other indications, the surgeon should ask parents for permission for prophylactic ablation. The prognosis of MD is good after excision.

90.5 Intestinal Obstructions

Intestinal congenital obstructions are caused by atresias and functional disorders of the gut:

- jejunal atresia
- ileal atresia
- colonic atresia
- meconium ileus.

90.5.1 Jejunal and Ileal Atresia

Represent 95% of congenital intestinal intrinsic obstructions. The remaining 5% are due to intestinal stenosis.

The most common sites are:
- proximal jejunum (31%)
- distal jejunum (20%)
- proximal ileum (13%)
- distal ileum (36%).

Incidence varies from 1 in 1500–2000 births and therefore rather more common than duodenal atresia. Usually these babies are low weight at birth.

90.5.1.1 Classification

Five different types [18]:
- Type I: No apparent external interruption to the bowel at the level of the atretic loop, but dilatation above the endoluminal mucous web.
- Type II: The atretic bowel is joined to the subsequent segment by a thin fibrous cord with intact mesentery.
- Type IIIa: The atretic segments are completely separated with a "V"-shaped defect of the mesentery.
- Type IIIb: There is an "apple peel" (sometimes known as "Christmas Tree") defect of the mesentery (Fig. 90.5).
- Type IV: Multiple atresias.

The most frequent is the type IIIa. The most serious types are IIIb and IV.

90.5.1.2 Embryogenesis

In contrast to other intestinal atresias (esophageal, duodenal, rectal) that have their origins during early development (embryopathies), jejunal and ileal atresias develop later (fetopathies). For this reason they are rarely associated with other anomalies and usually are only apparent after the fifth month of gestation. Two theories that have been proposed to explain the genesis of this malformation:
- Louw-Barnard theory (1955): This states that atresias are the consequence of ischemic bowel damage that may have another cause (e.g., volvulus, internal hernia, vascular disturbance), resulting in necrosis of the intestinal segment with consequent reabsorption and creation of an atretic pouch. This theory seems more likely than the alternative theory.
- Tandler theory (1902): This proposes an error during the phase of recanalization that follows the solid phase of intestinal development causing atresia.

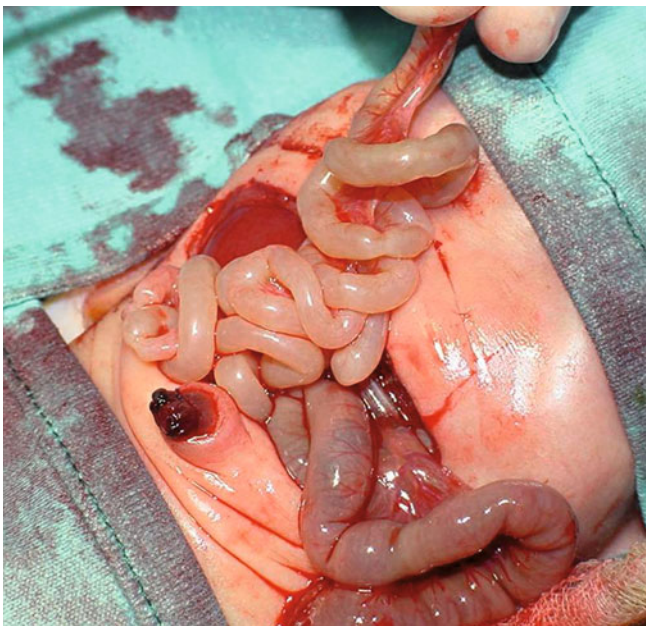


Fig. 90.5 "Apple peel" ileal atresia

90.5.1.3 Associated Anomalies

Intestinal atresias are usually isolated anomalies. Sometimes associated with other malformations (e.g., gastroschisis, omphalocele, meconium peritonitis) and probably secondary to ischemic damage of the bowel caused by these conditions.

A familial pattern of multiple atresias affecting the stomach, duodenum, small bowel and colon has been described in individuals of French-Canadian background, probably representing a rare autosomal recessive gene.

90.5.1.4 Antenatal Diagnosis

Antenatal diagnosis is possible after the 20th week of gestational age. Often there is associated polyhydramnios, usually more pronounced the higher the position of the atresia in the intestinal tract. Antenatal ultrasound shows multiple "bubbles" representing intestinal loops filled with amniotic fluid and secretions and apparent after the 24th week of development.

These findings are not pathognomonic of intestinal atresia and can also be found with other conditions, e.g., meconium ileus, total colonic atresia, Hirschsprung's disease, small left colon syndrome, meconium plug syndrome.

90.5.1.5 Symptoms and Diagnosis

In the absence of an antenatal diagnosis, the newborn will have progressively severe abdominal distension, followed by bile-stained vomiting.

A plain abdominal X-ray shows multiple air-fluid levels and no gas beyond the atresia (Fig. 90.6). The more distal the atresia, the greater the abdominal distension and the greater the number of distended intestinal loops and air-fluid levels. The abdominal radiograph should also be carefully examined for signs of intra-abdominal calcification, which is pathognomonic of meconium peritonitis.

A contrast enema with gastrographin should be done to investigate a possible colonic atresia. It may suggest Hirschsprung's disease. It may also be therapeutic if there is a meconium ileus or meconium plug syndrome and about

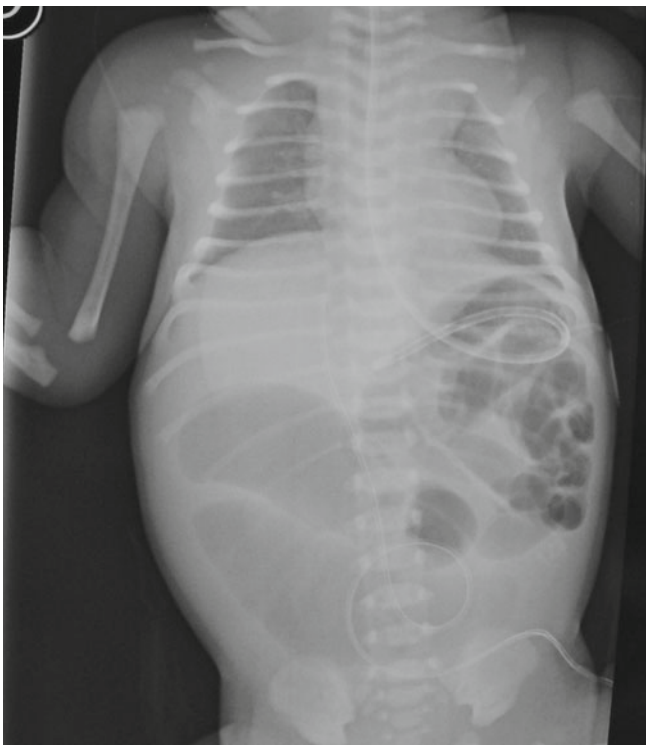


Fig. 90.6 Abdominal X-ray showing the ileal distension and no gas in the large bowel

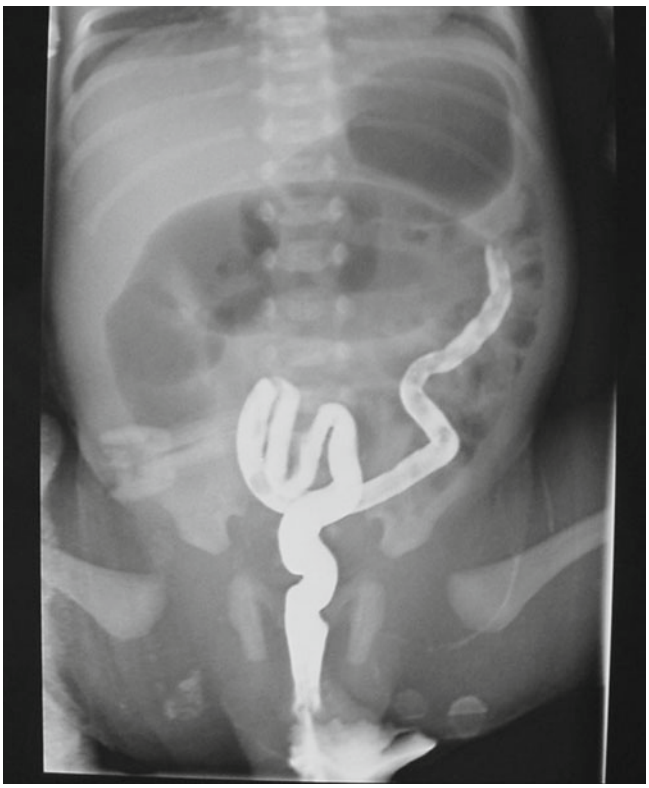


Fig. 90.7 Contrast enema showing the microcolon and the "soap bubbles" image typical of Meconium ileus

50% of patients with uncomplicated meconium ileus respond to such non-operative therapy (Fig. 90.7).

Meconium ileus and 10% of ileal atresias may be consequent to cystic fibrosis and these infants should have sweat tests before hospital discharge.

90.5.1.6 Therapy

These are not surgical emergencies and surgery can be delayed until the 2nd or 3rd day of life, after placing a nasogastric tube and starting total parenteral nutrition. The standard surgical approach is by a transverse supraumbilical laparotomy. However, these days a video-assisted approach is used and laparoscopy using a transumbilical approach allows for exteriorisation of the atretic loop through the umbilical incision (which can be enlarged laterally by 1–2 cm).

- In types I, II and IIIa, a primary end-to-end anastomosis is performed.
- In type IIIb a primary anastomosis is usually performed or, a temporary ileostomy can be fashioned with the anastomosis being delayed for several months.
- In type IV, primary anastomosis is done.

Total parenteral nutrition is continued until there are bowel movements and the gastric stasis ceases.

90.5.1.7 Prognosis

There is a good outcome in 90–95% of babies if other malformations are absent. Type IIIb has a poorer recovery rate due to a poorer gut blood supply.

"Short bowel syndrome" (SBS) may complicate type IIIb or IV atresias where a large section of bowel has to be removed. This is a serious complication, which may have dire consequences in terms of poor nutrition. However, some techniques have been developed to attempt to elongate the residual bowel to increase the absorptive surface of bowel (particularly, Bianchi's technique). The outcome is good for babies who have 25–30 cm of functioning small intestine and a preserved the ileo-caecal valve [19].

90.5.2 Colonic Atresia

Extremely rare. Above the splenic flexure in more than 50% of cases. When below and at the level of the sigmoid, may be confused with rectal atresia.

Incidence varies from 1 in every 10,000–20,000 births [20]. Only pyloric atresia is rarer than colonic atresia.

90.5.2.1 Classification

Similar to the classification used for the small bowel atresias:

- Type I: Endoluminal mucous web (complete or partial).

- Type II: A thin fibrous cord, with intact mesentery joining the two segments.
- Type III: The atretic segments are completely separated with a "V"-shaped defect of the mesentery.

90.5.2.2 Embryogenesis

Similar theories as for small bowel atresias.

90.5.2.3 Associated Anomalies

Skeletal malformations (syndactylism, polydactylism, radial agenesis) are fairly frequent, as are cardiac and ocular anomalies. Sometimes seen in patients with an omphalocoele or gastroschisis, and probably a consequence of ischemic damage due to the pathology itself.

90.5.2.4 Symptoms and Diagnosis

Colonic atresia is very difficult to recognize by antenatal ultrasound. Mostly there is a normal liquor volume and the presence of dilated bowel loops is not pathognomonic.If no antenatal diagnosis has been made, there will be abdominal distension without the passage of meconium, followed by bile-stained vomiting.

A plain abdominal X-ray shows multiple air-fluid levels either in small or large intestine and no gas beyond that point.

A gastrographin contrast enema will show a micro-colon and allow for the recognition of colonic atresia, or raises the suspicion of Hirschsprung's disease and may be therapeutic if there is meconium ileus or meconium plug syndrome.

90.5.2.5 Therapy and Prognosis

Not a surgical emergency, as for small bowel atresias. An umbilical incision and video assisted approach allows the dilated colon to be exteriorised and primary colo-colonic anastomosis. If the proximal colon is very dilated, a temporary colostomy is indicated, delaying the definitive colo-colonic anastomosis for weeks or even months. Colonic biopsies must be taken for the histological identification of neuronal cells and to exclude Hirschsprung's disease.

There is a good outcome in more than 90% of babies if no other malformations.

90.5.3 Meconium Ileus

A congenital neonatal intestinal obstruction due to thickened and tenacious meconium that cannot pass along the intestinal lumen and accumulates within it. Due to an autosomal recessive disease, cystic fibrosis (CF), which is characterized by various clinical manifestations of which meconium ileus (MI) is an early and serious one.

Between 1 in 1500–2000 births in white population. Rare in African-Caribbean babies (1/17,000) and extremely rare in those of Asian origin (1/100,000) [21]. A family history of CF is present in 10%–33% of patients with MI.

90.5.3.1 Embryogenesis

The defect of CF is an exocrine gland dysfunction, particularly of mucus secreting and sweat glands. Two pathological events in meconium ileus lead to the intraluminal accumulation of tenacious meconium: the development of a pancreatic exocrine enzyme deficiency and the secretion of hyperviscous mucus by pathologically abnormal intestinal glands

90.5.3.2 Antenatal Diagnosis

Family history, amniocentesis and antenatal ultrasound allow for the prediction of which infants are at risk of developing MI (almost 20% of CF population) [21].

90.5.3.3 Differential Diagnosis

The major differential diagnosis is with other causes of neonatal intestinal obstruction, in particular:

- ileal atresia
- meconium plug syndrome
- neonatal small left colon
- Hirschsprung's disease.

90.5.3.4 Symptoms and Diagnosis

MI presents during the newborn period in two forms:

- uncomplicated or simple MI
- complicated MI (meconium peritonitis).

Uncomplicated MI presents at birth and abdominal distension is the main symptom. There is then bile-stained vomiting and failure to pass stool. The clinical picture is similar to that of intestinal atresia.

Complicated MI is often recognized in utero by the observation of intra-abdominal calcification due to intestinal perforation following intestinal obstruction. Sometimes a cystic mass is palpable at birth.

The radiological picture shows an absence of air-fluid levels since the gas cannot form above the inspissated meconium, and a granular "soap bubble" appearance due to air bubbles intermixed within the sticky meconium (Fig. 90.7). In complicated MI, abdominal calcifications can be observed.

A contrast enema shows a microcolon.

The diagnosis is by a sweat test, which measures the amount of sodium and chloride in the sweat. A sweat chloride

concentration above 60 mEq/L is diagnostic of CF [18]. The definitive diagnosis is by genetic studies.

MI may occur in the absence of CF in term or preterm infants with pancreatic insufficiency due to other causes.

90.5.3.5 Therapy and Prognosis

Non-surgical treatment consists of trying to dissolve the inspissated intraluminal meconium with solubilizing agents either taken by mouth or by enema (Noblett technique) [22]. Gastrografin enema is the standard non-operative treatment. It is a hyperosmolar, water-soluble, radiopaque solution that draws fluid into the intestinal lumen helping to release intestinal meconium. Surgery remains the mainstay of treatment of MI. The initial procedure is laparotomy with an enterostomy with irrigation and ileal emptying of meconium. An alternative procedure is the formation of a temporary ileostomy to relieve the obstruction.

Survival is now 100% either with non-operative treatment or surgery. This should be compared with results in the 1960s when survival was 33% for the first 6 months. The prognosis depends on the course of CF and its complications.

90.6 Duodenal Obstructions

Duodenal obstruction can be "intrinsic" or "extrinsic".

Intrinsic obstructions are more frequent and comprise, in decreasing order of frequency:

- duodenal atresia
- duodenal web
- endoluminal diverticulum.

The extrinsic forms are rare and include, in decreasing order of frequency:

- annular pancreas
- congenital Ladd's bands (in intestinal malrotation)
- duodenal duplication
- preduodenal portal vein.

The clinical features are similar for intrinsic and extrinsic forms. Incidence varies from 1 in 3000–5000 births.

90.6.1 Duodenal Atresia

In 90% of cases it is located in the first or second portion of the duodenum near the papilla of Vater, usually (75-85%) beyond the outflow of Oddi's sphincter.

There are three different types [23]:

- 1st type: complete duodenal web, with mucosal and submucosal layer.
- 2nd type: two blind pouches joined by a thin, short fibrous cord with intact mesentery.
- 3rd type: the two duodenal pouches are separate and there is a mesenteric "V-shaped" defect.

90.6.2 Partial Intrinsic Obstruction

- Incomplete mucous web: there is a diaphragm with a central hole that can be primary (created at the same time as the web) or secondary (consequent to a perforation of the web due to the increased pressure).
- Endoluminal diverticulum: a membranous fold that extends inside the lumen as a "windsock". It may be initially only partly obstructive, but either duodenal or biliary tree obstruction increases progressively. If it goes beyond the Vater papilla, there may be biliary stagnation with the formation of gall stones.

90.6.3 Partial Extrinsic Obstruction

- Annular pancreas: partially or totally obstructive. It is an embryologic consequence of a persistent primitive ventral pancreas that normally rotates around the duodenum to form the head of the pancreas (Fig. 90.8) [23].
- Ladd's bands: found in patients with intestinal malrotation where the caecum is in the epigastric region. These bands connect the caecum and the right abdominal wall compressing the descending part of the duodenum.
- Preduodenal portal vein: unusual and due to the anomalous persistence of the anterior vitelline veins.

90.6.4 Embryogenesis

Thought to depend on a defect in the vacuolization process of the primitive gut between the eighth and the tenth week of gestation.

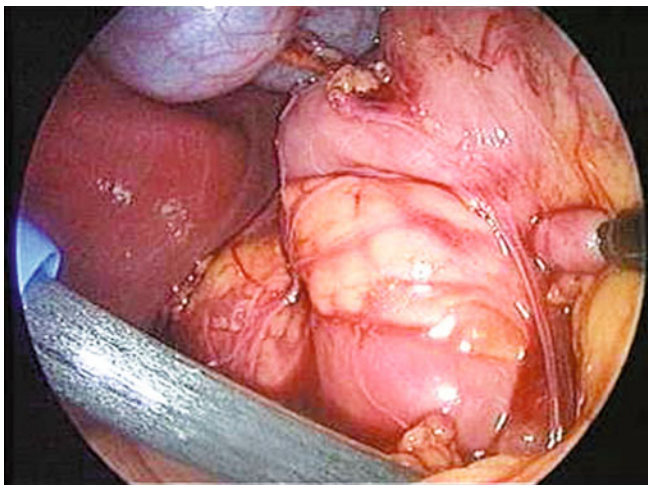


Fig. 90.8 Laparoscopic view of an annular pancreas

90.6.5 Associated Anomalies

Frequently associated with other congenital anomalies. The most frequent are skeletal (ribs, vertebrae, sacrum, radius) (36%), and intestinal (26%) (esophageal atresia, anorectal malformations, Hirschsprung's disease, malrotations). In 20% of cases, there is a cardiopathy. Urinary anomalies are present in 8% of cases.

Down syndrome is particularly frequent in subjects with duodenal atresia (30%) and if duodenal atresia is suspected on antenatal ultrasound, fetal karyotyping is recommended.

90.6.6 Symptoms and Diagnosis

It is possible to make an antenatal diagnosis after the 20th week. The ultrasound observation of polyhydramnios with a "double bubble" is diagnostic (except in the extremely rare case of coexisting type I esophageal atresia). If there has been no antenatal diagnosis, the main symptom is bile-stained vomiting in 80% of cases (due to the subvaterian atresia).

Plain abdominal radiography shows a diagnostic "double bubble" sign. If there is doubt about the diagnosis, a contrast medium will reveal the atresia or partial duodenal obstruction.

90.6.7 Therapy and Prognosis

Not a surgical emergency. The operation can be delayed for 24–72 hrs, allowing for pre-operative assessment, which should include studies of cardiac function.

After a nasogastric tube is passed, enteral feeding is replaced by total parenteral nutrition (TPN) and surgery for very low birth weight babies can be delayed until they have achieved an adequate weight.

- If there is a web, a duodenotomy is performed and the duodenal web is excised with careful attention to the Vater papilla.
- If there is atresia, a duodeno-duodenotomy is performed. The anastomosis is done using the Kimura technique where a "diamond-shaped" suture is used with a horizontal incision on the upper pouch and sagittal incision on the lower pouch (Fig. 90.9a,b) [24]. If the upper duodenum is dilated, "tapering" can be performed.

No gastrostomy is required. Oral feeding can be started when gastric aspirates are no longer bile-stained and bowel movements have started. This may take ten days or more.

The same operation is performed for an annular pancreas with particular attention to the choledocal and pancreatic ducts.

Laparoscopy has recently been used for duodenal atresia or stenosis [25]. Since this is a very difficult operation with limited workspace in the newborn abdominal cavity, a video-assisted approach allows for the identification of the two duodenal pouches, which are exteriorized through the umbilical incision and surgery is completed by traditional means.

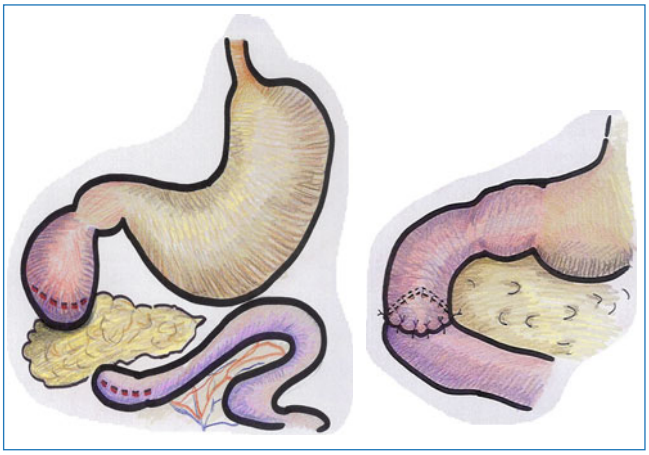


Fig. 90.9 The "diamond-shaped" Kimura's anastomosis

There is a 5% mortality due to associated malformations (mainly cardiac). Such operations should be performed a level III Centre with a neonatal ICU.

90.7 Pylorus

90.7.1 Pyloric Atresia, Aplasia

These malformations are very rare (1/100,000 births) without sex prevalence. There are fewer than 200 cases in the literature. These are often babies with more than one malformation, i.e., associated esophageal atresia, cardiac malformations.

The diagnosis can be made by antenatal ultrasound scanning after the 3rd–4th month of development when a single gastric bubble and polyhydramnios is seen. The neonate presents with gastric vomiting (i.e., no bile-staining) and epigastric distension. If there is an incomplete pyloric web, clinical features of obstruction occur later and it can be confused with hypertrophic pyloric stenosis [26]. The diagnosis is made by imaging using ultrasound and contrast medium radiography of the stomach.

Surgical resection of the obstruction is required, except when there is a membranous pyloric web that may be treated by endoscopic resection. In the last decade, a transumbilical skin incision with or without laparoscopy has become the surgical approach of choice for many procedures in neonates including pyloric pathologies.

90.7.2 Hypertrophic Pyloric Stenosis

Hypertrophic pyloric stenosis most frequent cause of gastrointestinal obstruction in children of all ages with an incidence

of about 0.2% and much more common in boys (male:female ratio 5:1).

No clear causative factors have been identified. Both genetic (ethnic variability, male preponderance and positive family history with first-born infant prevalence) and environmental (breastfeeding, seasonal variability with spring/autumn prevalence) factors seem to play a role. Histologically, there is marked hypertrophy of the circular muscle layer with complete reduction of the pyloric lumen. The pylorus is enlarged and elongated with a pale firm mass (pyloric "olive") measuring 2–2.5 cm in length and 1–1.5 cm in diameter.

90.7.2.1 Symptoms and Diagnosis

The onset of symptoms is at 2–8 weeks of age. They consist of non-bilious projectile vomiting (sometimes with blood), becoming progressively more severe and causing a hypochloraemic hypokalaemic alkalosis [27]. A delay in the diagnosis leads to serious dehydration.

In the past, the diagnosis was made by the clinical picture and palpation of the pyloric "olive" on the right side of the abdomen. Nowadays, ultrasound imaging frequently enables an accurate diagnosis (pyloric muscle thickness more than 3.5 mm, pyloric channel length more than 16 mm) [2]. If there is uncertainty, upper gastric contrast radiography enables a definitive diagnosis.

90.7.2.2 Therapy and Prognosis

Preoperative preparation includes stopping enteral feeds, passing a naso-gastric tube and intravenous fluids for correction of dehydration and electrolyte disturbances.

Surgical treatment is by Fredet-Ramstedt pyloromyotomy. The traditional approach was by a small right upper quadrant transverse incision, but in recent years a supraumbilical skin incision (Bianchi's technique) with the upper division of the midline fascia (Fig. 90.10) has been used [28].

After exteriorisation of the pyloric mass, a longitudinal sero-muscular incision of the pylorus is made. The muscle is split and separated till the mucosa bulges through the incision. In some centre this procedure is performed by laparoscopy [29]. Feeding may be restarted 6 hours after the operation, first with a glucose solution and then with increasing volumes of milk, reaching full feeds after 15–20 hours. Babies are discharged on the 3rd postoperative day.

Prognosis is excellent. Recurrences are unusual.

90.8 Defects of the Abdominal Wall

Defects of the anterior abdominal wall have a wide spectrum of presentation. They may extend to the thorax (Ectopia cordis) or present as an infraumbilical defect (exstrophy of the bladder). However, they are mostly limited to the umbilical region as an omphalocoele or gastroschisis.

90.8.1 Omphalocele

An omphalocele is central defect of the abdominal wall in the umbilical region, through which viscera may herniate. The defect is covered by a sac formed by the amniotic membrane on the outside and the peritoneum on the inside. The umbilical cord is always attached at the top of the sac (Fig. 90.11).

The size of the defect may vary from 4 to 8–10 cm. When the defect is wide, the sac may contain liver, small and large intestine and sometimes stomach, bladder and internal genitalia (uterus ovaries and adnexa in females; intra-abdominal testes in males). The larger the defect, the less well developed the abdominal cavity.

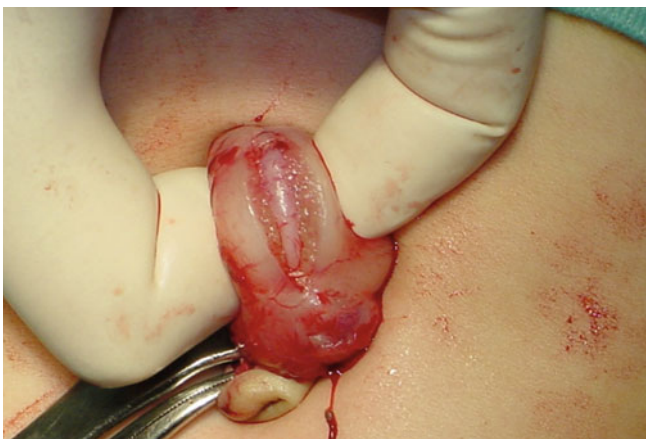


Fig. 90.10 Transumbilical pyloromyotomy. The muscle has been divided and split and it is possible to observe the bulging of the mucosa

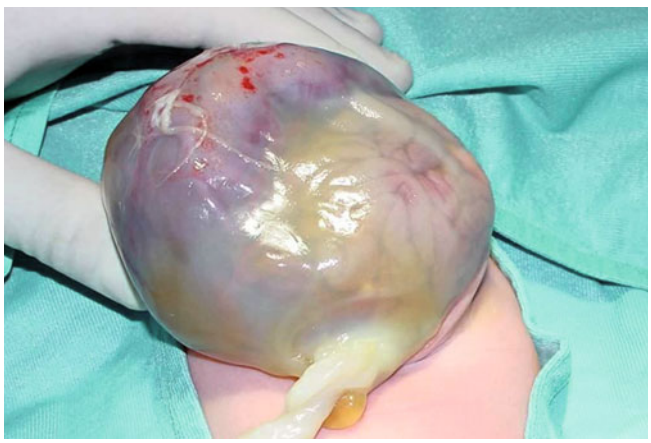


Fig. 90.11 Giant omphalocele. The liver and the intestinal loops are seen inside the translucent sac. Please note the cord insertion

The overall incidence is about 1 in 5000 live births with a slight male preponderance.

90.8.1.1 Embryology

During embryological development of the abdominal cavity, at about 5 weeks, the gut develops within the umbilical celom outside the abdominal wall and returns to the peritoneal cavity five weeks later. This is followed by rotation and fixation. Failure of this process causes a defect in the abdominal wall [30]. Since the gut is normally outside the peritoneal cavity until the 10th week of gestation, an omphalocoele cannot be diagnosed until the 12th week.

90.8.1.2 Associated Anomalies

About 50% of patients with omphalocele present with other associated anomalies. Most are cardiac anomalies (Tetralogy of Fallot, ventricular and atrial defects, ectopia cordis, and coarctation of the aorta). Chromosomal anomalies such as Down syndrome are more frequent in patients without herniation of the liver and have been reported in 15–20% of cases. The most frequent is the Beckwith-Wiedemann syndrome (7%), presenting as macroglossia, gigantism, visceromegaly, hypoglycemia and subsequent tumor formation, such as Wilms' tumor, hepatoblastoma, rabdomyosarcoma, neuroblastoma. Rarely the Beckwith-Wiedemann syndrome may be familial.

Omphalocole always presents with an intestinal malrotation due to incomplete intestinal rotation and fixation, and there is an increased incidence of volvulus.

90.8.1.3 Diagnosis

Antenatal diagnosis is possible after the 12th week of gestation, when the bowel returns to the peritoneal cavity. The ultrasound visualization of viscera outside the abdomen and contained by an amniotic sac with the umbilical cord at the top of the sac allows differentiation from gastroschisis.

90.8.1.4 Therapy Prognosis

Consider delivery at a level 3 perinatal center. After birth, the baby's abdominal wall should be covered with a tepid, moist gauze and wrapped in a plastic bag to avoid dehydration. A nasogastric tube is passed to drain the stomach and this may reveal an esophageal atresia. The herniated viscera is returned to the abdominal cavity and defect is closed, preferably at a single operation (primary closure).

For small omphaloceles, primary closure is almost always achieved. The sac is excised and the bowel reinserted in the abdomen. Often "stretching" the abdominal muscular wall is required to close the defect without increasing intra-abdominal pressure to such an extent that the cardiopulmonary system is compromised by reducing venous return [31].

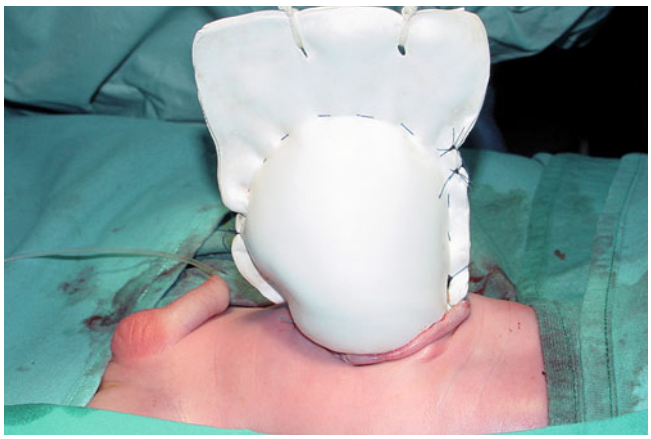


Fig. 90.12 Giant omphalocele. A silastic prosthesis has been sutured to the abdominal wall according to the Schuster's technique. It will be progressively reduced in the following days

If the omphalocele is so large that primary closure is not possible, a staged reduction of the intestinal contents using a silastic prosthesis (Schuster's technique) is used (Fig. 90.12) [32]. The viscera are placed in a silo and are gradually squeezed back into the abdomen until complete closure of the abdominal wall is achieved after a couple of weeks.

Dependent on associated conditions, in particular the presence of cardiac malformations, and on abdominal complications related to the intervention and to the use of the prosthesis, e.g., infections, intestinal adhesions and intestinal obstruction. Survival rate is 75–80%.

90.8.2 Gastroschisis

A small (2–4 cm) defect of the abdominal wall to the right of the abdominal insertion of the umbilical cord, through which intestinal loops herniate uncovered by any sac or membrane (Fig. 90.13). The liver is always inside the abdomen. The bowel may be thickened and edematous because of the chemical action of amniotic fluid and kinking of the mesenteric vessels; less frequently, the bowel appearances are normal without any sign of inflammation.

The overall incidence is about 1 in 10,000 live births with a slight male preponderance. The incidence is highest in Northern Europe [30].

90.8.2.1 Embryology

A likely consequence of intrauterine regression of the right umbilical vein, determining the development of a site of least resistance through which the bowel protrudes before complete fixation.

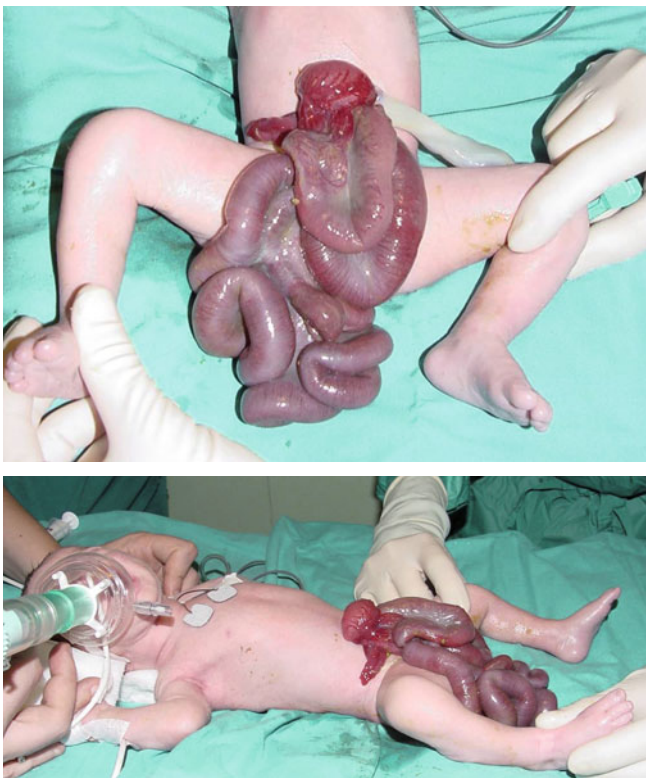


Fig. 90.13 Gastroschisis. The defect of the abdominal wall is on the right of the umbilical cord insertion

90.8.2.2 Associated Anomalies

Since gastroschisis occurs late during development, the incidence of associated anomalies is low. Intestinal malrotation is almost always present. In 10–15% of cases, there is intestinal atresia or stenosis, probably because of ischemic bowel injury. Cardiac anomalies complicate 1% of cases. Chromosome anomalies are rare. These babies are often small for date because of excessive protein loss into the amniotic fluid.

90.8.2.3 Diagnosis

Antenatal diagnosis is possible after the 12th week of pregnancy. Ultrasound is used to visualize intestinal loops outside the abdomen, uncovered by an amniotic sac with normal insertion of the umbilical cord. There is often polyhydramnios. Delivery should be by cesarean section.

90.8.2.4 Therapy and Prognosis

The herniated bowel is covered by tepid, moist sterile gauze and wrapped in a plastic bag to prevent dehydration. A nasogastric tube is passed to deflate the stomach.

The first attempt at closure is by Bianchi's "minimal invasive management" (Fig. 90.14) [33]. This is not an operative procedure but consists of patient, slow manual reduction

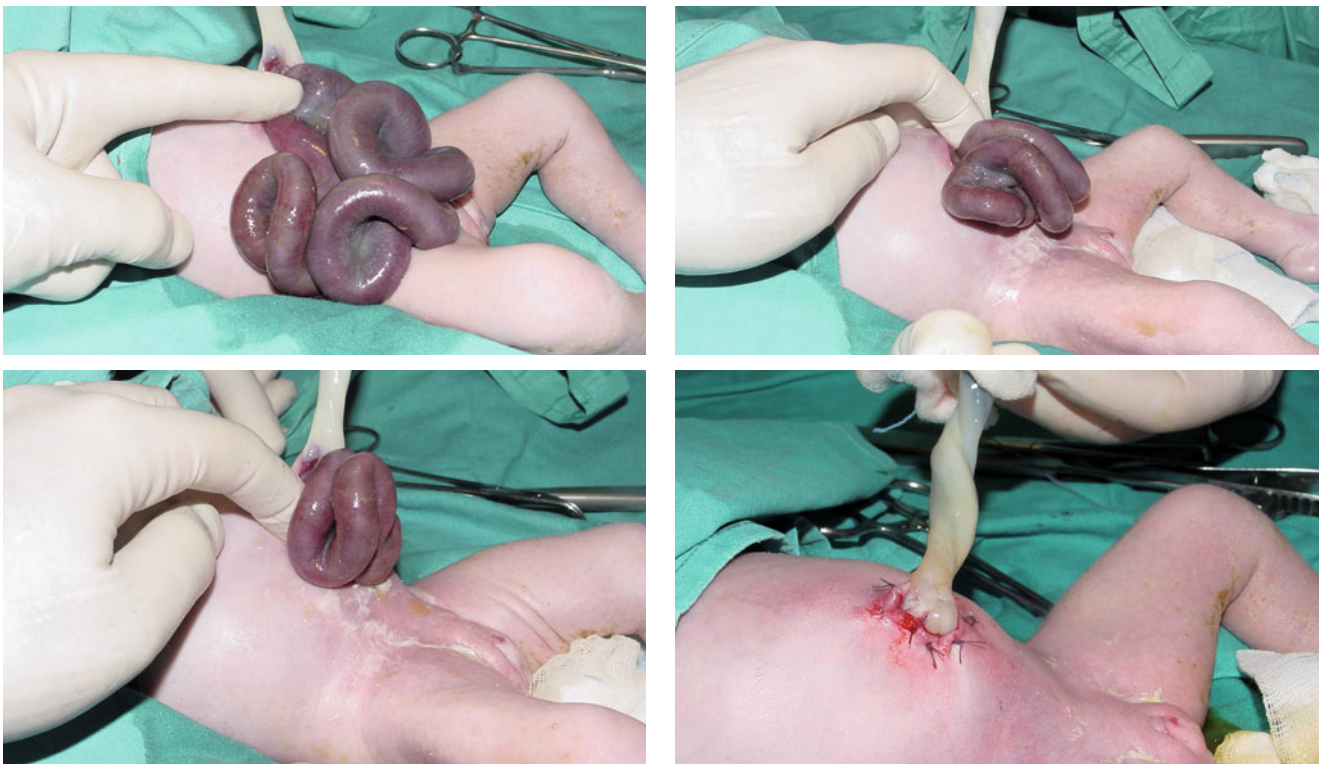


Fig. 90.14 Gastroschisis. Bianchi's "minimal invasive management"

of the herniated intestinal loops through the original hole. The maneuver is done with the baby asleep in his crib without general anesthesia and can be completed in 3–4 hours, leaving the bowel to adapt within the peritoneal cavity. This approach may be followed by prolonged absence of bowel motility and parenteral nutrition must therefore be started.

When reduction using this approach fails, usually because of serious thickening of the bowel wall, a traditional surgical operation is used where the defect is enlarged cranially and caudally by a few centimetres and the gut is re-introduced into the abdomen, sometimes using the "stretching" manoeuvre [34]. If reduction remains impossible, the staged Schuster's (silo) technique is used [32].

Survival rate is high, about 85–90%. Mortality is principally due to the low birth weight and associated intestinal conditions, e.g., atresia, stenosis, bowel perforation.

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91

Rare Surgical Emergencies

Mario Messina, Francesco Molinaro and Alfredo Garzi

91.1 Background

Over the past decade, multiple factors have changed the pattern of neonatal surgical emergencies. An increase in prenatal screenings and the development of neonatal tertiary care centers have changed the clinical approach to these kinds of emergencies. In fact, most conditions, leading to gastrointestinal (GI) emergencies, are uncommon and treatment in specialist centers enables concentration of appropriate resources and expertise. Co-morbidity is common, particularly in the preterm or low birth weight infant [1].

Symptoms of GI emergencies may be subtle, including irritability or feeding intolerance, or they may be more apparent, with vomiting (bilious or nonbilious), abdominal distention, and shock. Vomiting in the neonatal period should always prompt the consideration of a pathologic process. It may be difficult to differentiate a life-threatening cause from a mild viral gastroenteritis or even severe gastroesophageal reflux. The initial symptoms may be nonspecific and the history may not be helpful in a neonate who has not developed a normal pattern. Early diagnosis, availability of diagnostic service and prompt surgical intervention with optimal pre- and post-operative care are necessary to increase survival of newborns with such problems [2]. In this chapter we only explain the pathologies causing GI emergencies in newborns with a rare prevalence. Table 91.1 reports a list of the principal causes of neonatal surgical emergencies of the GI tract performed according to the current literature.

91.2 Incidence, Etiology and Pathophysiology

The principal causes of neonatal surgical emergencies of the GI tract can be divided into common and uncommon ones and can cause an occlusive or a perforative clinical pattern (Table 91.1).

91.2.1 Obstruction

Intestinal obstruction is the most common surgical emergency of the newborn. The incidence of neonatal intestinal obstruction is approximately 1 case per every 500–1000 live births. Approximately 50% of these neonates will have intestinal atresia or stenosis. Duodenal atresia and jejunal atresia occur in approximately equal numbers, although some authors report that jejunoileal atresia is the more common [3]. Congenital pyloric atresia (CPA) is a very rare condition that was first described by James Calder in 1734 [4]. Commonly, CPA occurs as an isolated lesion, which has an excellent prognosis, but it can also be seen in association with other malformations, which can have a negative impact on the final outcome [5]. Colon atresia is an extremely rare condition [6].

Intestinal duplications are rare congenital malformations that could cause intestinal obstruction as mass effect or causing volvulus. Duplications of the alimentary tract occur in 1 of 4500 births Gastric duplications constitute 8% of these or roughly 17 of every 1,000,000 births. A bowel volvulus caused by the presence of a cystic intestinal duplication can lead to an intestinal perforation. The different locations and sizes of these duplications require a specific diagnostic and surgical approach. Early diagnosis and treatment of uncomplicated intestinal duplications by means of prenatal sonographic screening and laparoscopic-assisted resection, respectively, are desirable in this congenital malformation, in order to prevent the risk of occlusion/perforation [7].

91.2.1.1 Pathophysiology

Proximal intestinal obstruction as in cases of duodenal or pyloric atresia leads to fluid loss that has a high concentration of hydrogen, potassium and chloride ions. Hypochloremic

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Table 91.1 Neonatal surgical emergencies of GI tract. The tables shows the principal causes of neonatal emergencies, specifying the common and uncommon ones

Occlusion	
Common	*Uncommon*
– Malrotation (duodenal obstruction, volvulus, internal hernia)	– Gastric duplications and acute stomach volvulus
– Duodenal atresia, stenosis or annular pancreas	– Congenital pyloric atresia or web
– Jejunal atresia or stenosis	– Duodenal duplication
– Ileal atresia or stenosis	– Preduodenal portal vein
– Simple meconium ileus	– Cystic duplication of the bowel with volvulus
– Meconium ileus with perforation	– Colon atresia
– Meconium plug syndrome	– Colon duplication with volvulus
– Hirschsprung's disease	– Functional intestinal obstruction
– Drug-induced ileus	– Intestinal psuedo-obstruction
– Hypertrophic pyloric stenos	– Neuronal intestinal dysplasia
– Meckel's diverticulum	
Perforation	
Common	*Uncommon*
– Necrotising enterocolitis	– Spontaneous intestinal perforation
– Meckel's diverticulum	
Miscellaneus	
Common	*Uncommon*
– Hemangiomas, lymphangiomas and vascular malformations of GI	– Neonatal appendicitis
	– Littre's hernia

alkalosis can develop if fluid losses are not replaced. Distal intestinal obstructions lead to fluid and electrolyte loss from both emesis as well as from fluid sequestered into the lumen of dilated bowel loops. Fluid shifts and intravascular volume depletion may lead to severe dehydration, oliguria, metabolic acidosis, and inadequate peripheral perfusion. Prolonged intestinal obstruction leads to alterations in intestinal motility, accumulation of gas and fluid, and bacterial overgrowth. Severe abdominal distention in the neonate can easily impair diaphragmatic function causing respiratory acidosis. As plasma volume loss increases, alterations in blood flow may result in bowel ischemia and necrosis [3].

91.3 Perforation

Gastrointestinal perforation is a catastrophic condition in neonates, especially in premature neonates.

Spontaneous perforations of the gastrointestinal tract occur in the stomach, duodenum, small intestine or colon. The problem is encountered only infrequently; so, the exact incidence is unknown. Male infants are affected more often than females (approx. 4:1). Although neonatal gastric perforation is a well-recognized entity, other spontaneous GI perforations are much rarer and often incorrectly attributed to other disease processes (i.e., necrotizing enterocolitis) [8].

Gastric perforation among neonates is a rare but frequently fatal condition of uncertain etiology. It is associated with high mortality, particularly in premature infants. There is also a trend towards higher mortality in lower-birth-weight infants [9].

91.3.1 Pathophysiology

Almost all spontaneous perforations of the GI tract are considered to be the result of ischemic necrosis. The perforation is the end result of "selective circulatory ischemia," a defense mechanism of the neonate to hypoxia, physiologic stress, and shock. Microembolic phenomena may also play a role. In response to physiologic stress (hypoxia, hypovolemia, etc.), blood is selectively shunted away from mesenteric vessels to the more vital heart and brain. Local mesenteric ischemia can progress to microvascular thrombosis and subsequent gastrointestinal wall necrosis and perforation. Although ischemia is likely the underlying problem, other factors including bacterial colonization, hyperosmolar feeds, and an immature neonatal immune system may also contribute. Indomethacin may also play a causal role in spontaneous GI perforations particularly in preterm infants as it does in the etiology of necrotizing enterocolitis. Risk factors for neonatal gastrointestinal perforation include all causes of severe fetal distress (abruption, emergent c-section, etc.) [8].

Specifically, in the gastric tract, gastric wall ischemia, hypothalamic mechanisms, excessive gastric acid-pepsin ulceration and congenital muscular wall defects could account for spontaneous gastric perforation, even if many authors believe

that this spontaneous rupture is due to an acute rise in the intragastric pressure at the time of parturition [9].

91.4 Miscellaneous

Acute neonatal appendicitis is a rare condition associated with significant morbidity and mortality. The severity of this disease is caused by its tendency to occur more frequently in premature infants, an increased perforation rate with rapid progression to peritonitis, and delay in diagnosis and intervention. Although appendicitis in the perinatal period may occur as an isolated event, in many cases it occurs in association with other pathologic states, including prematurity, inguinal hernia, and others. The presentation of neonatal appendicitis can be identical to necrotizing enterocolitis, leading to misdiagnosis [10].

Littre's Hernia was originally defined by Rinke in 1841 as "the presence of a Meckel's diverticulum in any hernial sac". It is generally difficult to differentiate from other types of hernia, until complications arise. It is a rare and accidental finding at any age, but absolutely exceptional in neonates. The potential surgical risk is linked to the presence of a Meckel's diverticulum [11].

91.5 Clinical Findings

91.5.1 Occlusion

Prenatal ultrasonography can readily identify proximal obstructing lesions that can produce proximal bowel dilation with hyperperistalsis and the classic "double bubble" appearance of duodenal atresia. Distal intestinal obstructions are less likely to cause polyhydramnios but on occasion dilated loops of bowel may be identified as anechoic masses.

Over the maternal polyhydramnios, four clinical findings suggest intestinal obstruction in the neonate: excessive gastric aspirant, abdominal distention, bilious vomiting, and failure to pass meconium. The presence or absence of each of these clinical findings depends largely upon the level of gastrointestinal obstruction. Early recognition of intestinal obstruction is imperative if the complications of respiratory compromise and sepsis are to be avoided [2].

91.5.1.1 Excessive Gastric Output

Passage of a nasogastric or orogastric tube is often performed in premature infants and infants with a maternal history of polyhydramnios. If the initial volume of gastric aspirant is large (> 50 cc) or is bilious then gastrointestinal obstruction should be considered.

91.5.1.2 Abdominal Distention

Abdominal distension may not be apparent at birth but develops over time as ingested air accumulates proximal to an obstruction. The time of onset, degree and characteristic appearance of the distention may suggest the level of obstruction. Gastric distention within a few hours may cause the epigastrium to protrude indicating an obstruction of the stomach or duodenum. Gradual overall abdominal distension occurring over a 12–24 hour period suggests a distal gastrointestinal tract obstruction.

91.5.1.3 Bilious Emesis

It is usual for healthy newborns to spit-up postprandially but bilious emesis in a term newborn is distinctly abnormal. Premature infants (< 35 weeks) occasionally have bilious emesis secondary to an immature or poorly functioning pyloric sphincter but proximal gastrointestinal obstruction must still be considered. Sepsis with an associated paralytic ileus may also result in bilious emesis. Vomiting begins soon after delivery if the lesion is proximal or complete, but may be delayed in cases of distal or incomplete obstruction.

91.5.1.4 Failure to Pass Meconium

A normal newborn is expected to pass a large amount of thick, dark green, shiny meconium usually within the first twelve hours of life and almost always by 24 hours. Failed or delayed passage of meconium suggests obstruction, but neonates with proximal obstructing lesions may pass a normal amount of meconium. Because neonates with ileal atresia or distal small bowel obstruction may pass several meconium stools on the first day of life, passage of meconium does not exclude the possibility of obstruction. Preterm infants commonly have delayed passage of meconium. Approximately 20% will not pass stool during the first 48 hours following birth.

Additional physical findings that suggest obstruction are the presence of intestinal patterning and peristalsis that is visible through the intestinal wall. Distended loops of bowel may be palpable as ill-defined tubular masses. The rectum may feel tight on exam if small and unused as in cases of distal bowel obstruction. Lethargy and hypotonia are late signs of intestinal obstruction and the resultant sepsis. Abdominal wall discoloration and ecchymosis suggest perforation and/or necrosis [3].

91.6 Perforation

Most infants can immediately present signs of perforation. In fact, in cases of spontaneous gastrointestinal perforation

infants present within the first week of life (usually 4–5 days) with an abrupt onset of abdominal distention and associated tachycardia, hypovolemia, and poor systemic perfusion. With severe pneumoperitoneum, respiratory function is compromised requiring urgent intubation. Typically, the abdomen is markedly distended and tympanitic to percussion. Pneumoperitoneum is usually present in these infants. The clinical course of neonates with spontaneous gastrointestinal perforation may mimic those of NEC or other diseases associated with perforation [8].

91.7 Diagnosis

In cases of rare disease causing emergencies, the diagnosis can be difficult, but it is possible to research clinical grounds of intestinal obstruction or perforation.

In case of occlusion, flat and upright abdominal radiographs are obtained to confirm the diagnosis. Swallowed air serves as the contrast media to help delineate the level of obstruction. A normal neonate swallows air from birth and has air within the proximal small bowel within 30 minutes. Air usually reaches the colon by 3–4 hours and can be identified in the rectum by 6–8 hours. There should be no air-fluid levels in an upright film of a normal newborn [12].

When a complete obstruction exists the air pattern may stop abruptly leaving the remainder of the bowel airless. Bowel loops proximal to complete obstruction are dilated. Multiple dilated loops of bowel with "stepladder" air fluid levels on the upright film is the pattern most often seen with distal intestinal obstruction. However, air-fluid levels are not characteristic of the distal intestinal obstruction due to meconium ileus. Partial obstructions as in stenosis may allow small amounts of air to pass beyond the level of obstruction, but the paucity of bowel gas in the bowel distal to dilated bowel segments can easily be identified as abnormal. The abdominal films should always be inspected for peritoneal and/or scrotal calcifications which may signify an intrauterine perforation with meconium peritonitis.

Barium or gastrografin contrast enema examination may be useful to distinguish between causes of distal bowel obstruction (ileal atresia, meconium ileus, Hirschsprung's, meconium plug, etc.) and in cases of meconium ileus or plug may also be therapeutic. Upper gastrointestinal barium studies are generally not as useful unless one is seeking patterns of abnormal bowel rotation. UGI contrast studies are reserved for cases of partial obstruction that cannot be confirmed by plain radiographs [13].

Under the suspicion of perforation the contrast study is strictly not recommended and the diagnosis is confirmed by plain abdominal X-rays (flat and decubitus views) that demonstrate free intraperitoneal air. Serial abdominal films are to be obtained if perforation cannot be demonstrated initially but remains highly suspected [8].

Additional laboratory evaluation includes blood cultures, blood leukocyte and platelet counts, arterial blood gases, and serum pH, especially in case of appendicitis.

91.8 Treatment

The specific management strategies and surgical considerations for the various conditions causing intestinal obstruction in the neonate are not described in this chapter. However, the initial treatment of any suspected neonatal obstruction includes placement of a nasogastric tube to decompress the stomach and to prevent vomiting/aspiration. Fluid and electrolyte replacement should be quickly undertaken to resuscitate the infant and restore circulating blood volume in anticipation of the potential need for surgical intervention. Most obstructive lesions in neonates will require surgical therapy and surgery should not be delayed once volume resuscitation is adequate. If an intestinal anastamosis is anticipated perioperative antibiotics are indicated [3].

In the presence of clinical and radiological signs of perforation, treatment commences as soon as possible, simultaneous to the diagnostic workup. Rapid deterioration is anticipated and prevented with aggressive fluid resuscitation, intravenous antibiotics, correction of acid-base disturbances, and nasogastric decompression. Intubation and ventilatory support is required in infants with respiratory distress. Aspiration of the massively distended pneumoperitoneum can be helpful in infants with severe life-threatening respiratory compromise. Surgical exploration is indicated. The site of perforation is identified although in up to 10% of cases the perforation site has sealed spontaneously and cannot be identified. Surgical treatment is dictated by the infants physiologic condition and the findings at laparotomy (i.e., site of perforation, tissue condition, soilage, etc) and include primary repair, resection with external diversion, resection with anastamosis, drainage, etc. Obstruction distal to the site of perforation is excluded whenever possible [14].

91.9 Outcomes

A multi-disciplinary team of surgeons, anesthetists, neonatologists, radiologists, cardiologists, obstetricians, nurses, physiotherapists and other health professionals experienced in dealing with extremely small infants will provide the best outcome.

The outcomes from neonatal intestinal obstruction or perforation vary with the etiology of the disease. Overall survival is generally good but often is influenced by the associated anomalies of each condition [2].

The prognosis is adversely affected by prematurity, the presence of other anomalies, and a delay in diagnosis [14].

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Meconium Plug Syndrome

Mario Messina and Francesco Molinaro

92.1 Definition

Meconium plug syndrome, also described as functional immaturity of the colon, is a transient disorder of the newborn colon characterized by the delayed passage (>24–48 h) of meconium associated with intestinal dilatation. The retained meconium shows up on contrast enema as a filling defect or plug producing a double-contrast effect. Small left colon syndrome is a subset of the meconium plug syndrome where an enema demonstrates an apparent transition zone between dilated distal colon and colon of normal or decreased calibre at the splenic flexure [1].

Fig. 92.1 Inspissated meconium causing colonic obstruction

92.2 Background

Meconium is the material found in the intestine in a newborn. It consists of succus entericus, which is made up of bile salts, bile acids, and debris shed from the intestinal mucosa during intrauterine life. It is normally evacuated within 6 hours after birth or in utero as a vagal response to perinatal stress [2]. The terms small left-colon syndrome, colon immaturity, and functional colonic obstruction are synonymous. All refer to functional immaturity of the ganglion cells of the colon, which manifests as failure to pass the first stool. The problem is probably more common than generally realized and, in most cases, the symptoms are so mild and transient that they pass unnoticed. However, if significant obstruction develops, symptoms become more serious and there may even be perforation [3].

The term meconium plug syndrome was first reported by Clatworthy in 1956 to describe colonic obstruction due to inspissated meconium (Fig. 92.1). Initial reports described a 13% incidence of Hirschsprung's disease with meconium plug syndrome and no association with cystic fibrosis [4]. However, the literature over the last 40 years has reported Hirschsprung's disease in as many as 38% and cystic fibrosis in as many as 43% of patients with meconium plug syndrome [1].

In a review, Keckler et al found that when meconium plug syndrome was identified as pellets only in the colon, cystic fibrosis was rare and the incidence of Hirschsprung's disease was around 13% [5].

There are three associated gastrointestinal (GI) conditions: meconium ileus, meconium peritonitis and ileus–equivalent syndrome.

92.3 Meconium Ileus

Meconium ileus occurs when meconium becomes inspissated and obstructs the distal ileum. The condition is usually a manifestation of cystic fibrosis and about 20% of infants with cystic fibrosis present with meconium ileus at birth.

Meconium ileus is generally the earliest manifestation of cystic fibrosis until proven otherwise. Meconium ileus

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may occasionally occur in association with pancreatic atresia or stenosis of the pancreatic duct. Meconium ileus also rarely occurs in the absence of any apparent abnormality of the pancreas and these infants have a favorable outcome after decompression because they do not have cystic fibrosis. The problem is more likely related to gut immaturity with decreased intestinal motility and impaired passage of meconium.

In meconium ileus, low or distal intestinal obstruction results from the impaction of thick, tenacious meconium in the distal small bowel. There may be additional complications, such as ileal atresia or stenosis, ileal perforation, meconium peritonitis and volvulus with or without pseudocyst formation [1].

92.4 Meconium Peritonitis

Meconium peritonitis usually results from intrauterine GI perforation. It may occur as early as the second trimester. It is initially a sterile chemical peritonitis, but if perforation of the GI tract persists after birth, complicating bacterial infection may supervene and the prognosis worsens. Perforation and subsequent meconium peritonitis have multiple causes, but most often are due to obstruction and/or volvulus associated with meconium ileus (and cystic fibrosis). In other instances, perforation is due to other causes of neonatal bowel obstruction (such as intestinal atresia) [1].

92.5 Meconium Ileus–Equivalent Syndrome

Meconium ileus–equivalent syndrome is a misnomer that is applied to a partial bowel obstruction in older patients known to have cystic fibrosis. Many prefer to call this the distal intestinal obstruction syndrome (DIOS). DIOS has nothing to do with meconium (as meconium is seen only in the newborn bowel), but on occasion the appearance of feces on the abdominal radiograph have been likened to that of meconium. In 15% of adolescents and adults with cystic fibrosis, abnormally viscid bowel contents become impacted in the distal small bowel.

It is estimated that 37% of patients with cystic fibrosis experience meconium ileus–equivalent syndrome or DIOS at some time [6].

92.6 Age

Although meconium plug syndrome is found mostly in term infants, Mees et al reported that three of their four patients were premature [4].

92.7 Clinical Details

92.7.1 Clinical Signs of Meconium Plug Syndrome

Meconium plug syndrome is common in infants of diabetic mothers (incidence 40%–50%), but also occurs in infants with neurological depression at birth or who are hypotonic for other reasons and hypermagnesemia has been implicated as a cause. Sepsis can also lead to diminished bowel motility (paralytic ileus), mimicking meconium plug syndrome. Most infants with this form of colonic obstruction present during the first 24–36 hours of life. Findings include abdominal distension, bile-stained vomiting, and failure to pass meconium.

The clinical presentations of meconium plug syndrome and meconium ileus are similar, although the source of meconium obstruction is different: ileal obstruction with proximal small bowel dilatation and microcolon in meconium ileus, while the meconium is located in the colon in meconium plug syndrome.

Hirschsprung's disease is also a cause of meconium plugging. The initial clinical and radiologic findings may be similar, but during an enema, abnormal contractions with bowel wall irregularity may be seen in the narrowed, aganglionic bowel of Hirschsprung's disease. The bowel wall typically has a smooth contour when there is an uncomplicated meconium plug [7].

92.7.2 Clinical Signs of Meconium Ileus

Infants with meconium ileus present with vomiting (usually bile stained), abdominal distension and a failure to pass meconium. Pulmonary abnormalities due to cystic fibrosis do not usually present at birth. Although meconium ileus in the absence of cystic fibrosis is considered rare, it occurred in 21.6% of newborns in one series and these infants had no laboratory or clinical evidence of cystic fibrosis [8].

92.7.3 Clinical Signs of Meconium Peritonitis

Meconium peritonitis may be incidentally detected on abdominal radiographs. Clinically, patients may present because of bowel obstruction caused by fibro-adhesive bands, which are the result of the inflammatory peritoneal reaction. The bowel may be intact with the perforation having healed, but there may be associated bowel atresias. If the processus vaginalis is patent when the perforation occurs, calcification or hernias may involve the scrotum. Ascites may also be present [9].

92.8 Preferred Examination

The preferred initial imaging modality is a plain film, which includes supine and horizontal beam views (left lateral decubitus or cross-table lateral) of the abdomen. Plain images are nonspecific and usually show findings of a low small-bowel obstruction. If an erect abdominal radiograph is done (seldom necessary in the newborn), there is a paucity of air-fluid levels, and, in most cases, no gas in the colon. If air is introduced into the rectum from below (e.g., by rectal examination), rectal gas may be visualized. In this situation, meconium in the rectum can erroneously suggest a small presacral mass. However, if air entirely surrounds the meconium mass, it will outline the contour of the mass and suggests the correct diagnosis.

If peristalsis forces gas from the small bowel into the colon, it mixes with meconium and there may be a granular or bubbly appearance, mimicking the findings of pneumatosis cystoides intestinalis and necrotizing enterocolitis (NEC). If a bubbly pattern is seen during the first 12 hours of life, meconium plug syndrome should be considered, but if it arises after 12-18 hours, NEC is more likely. NEC typically affects preterm infants.

Individual assessment is most important. A contrast enema shows a characteristic appearance of the colon, with contrast material outlining the solid column of inspissated meconium and the wall of the colon, giving a double-contrast effect.

Water-soluble contrast enemas can be curative, causing passage of the meconium. After the meconium has been passed, the part of the colon from which it was evacuated may appear narrowed, and a transition zone mimicking that seen in Hirschsprung's disease may be seen. The small, contracted portion of the descending colon in these infants has led to the term, neonatal small left-colon syndrome [7].

92.9 Management

The treatment of meconium plug or small left-colon syndrome is conservative. It consists of stimulation of the rectum or colon. In most cases, simple finger examination of the rectum or insertion of a rectal thermometer produces enough stimulation to induce peristalsis and promote evacuation of the meconium. In other cases, a water-soluble contrast enema examination may induce meconium passage. If this or saline enemas fail, water-soluble contrast agents, should be used. Examinations with such contrast materials require careful attention to the patient's fluid and electrolyte balance [10]. With conservative treatment, complete decompression usually occurs within hours. However, in some infants, the process may take up to 2–3 days.

92.10 Contrast Enema Technique

- Use blankets and lamps to keep the baby warm and the contrast medium should be warmed to body temperature.
- Advance a small-caliber soft, rubber catheter (10–14F) into the rectum using minimal lubricant. Hold the catheter in place by taping the patient's buttocks tightly together.
- Some radiologists deploy a small Foley balloon (5 mL) to aid contrast retention. The balloon should not be inflated until after a preliminary injection of a small amount of contrast under fluoroscopy demonstrates adequate caliber of the rectum. This initial injection without an inflated balloon also allows assessment for a possible low-transition zone in patients with Hirschsprung's disease.
- Because the internal volume of the colon may be low, at the author's institution contrast is injected by hand using a syringe under careful and almost continuous fluoroscopic monitoring. Others use a low-capacity reservoir, such as an intravenous bag, and infuse via gravity drip.
- The entire colon should be opacified to maximize the therapeutic effect of the enema and to identify the position of the caecum (Fig. 92.2).
- This should be followed by carefully enabling reflux of the contrast medium into the distal ileum in patients who may have meconium ileus.
- After the colon has been filled and appropriate films obtained, the catheter is removed without attempting to drain the colon. Allowing the patient to expel the contrast may dislodge the meconium plug [11].

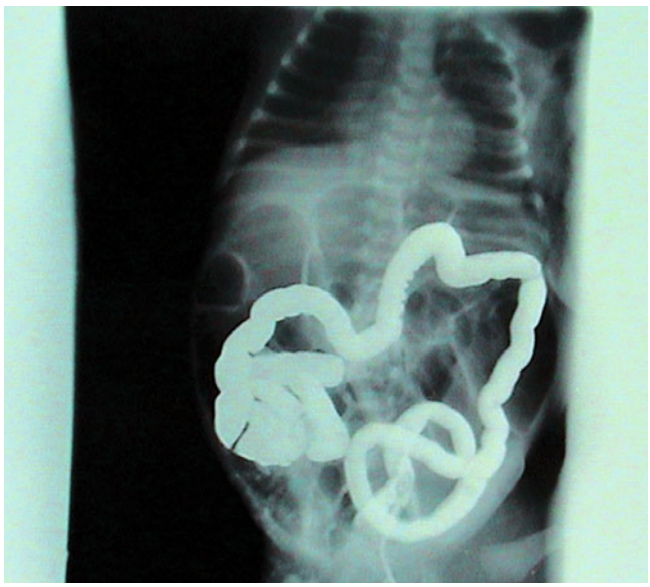

Fig. 92.2 The entire colon should be opacified both to maximize the therapeutic effect of the enema and to identify the position of the caecum

Response to the enema is often dramatic and curative, with immediate passage of meconium and resolution of intestinal dilatation. In some patients, clinical findings persist and a second therapeutic enema may be required. However, since the plug is a symptom in this disorder rather than its cause, multiple repeat procedures are not generally justified [12].

About half of neonates with meconium ileus cannot be treated adequately with irrigations and/or additionally have an intestinal obstruction complicated by neonatal intestinal perforation or ileal atresia secondary to intrauterine perforation. Such cases always require a surgical procedure such as resection of the dilated meconium-filled ileum and ileal anastomosis. Complicated cases of meconium ileus are seen in newborns with an extreme difference in diameters of the proximal and distal ileum, and a significant microcolon. If there is an atresia, the atretic segment should be resected and thick meconium from the proximal part and the grey stool pellets from the distal part evacuated by repeated irrigations with warm saline through a 5–8 Ch feeding tube with gentle manual forward and backward manipulation [13].

A variety of surgical techniques have been developed, comprising resection of the enlarged bowel segment and temporary decompression by distal or proximal enterostomy. The simplest form is a double-barreled ileostomy, described by Mikulicz, with the two loops brought out side-to-side. This is quick and avoids an intra-abdominal anastomosis. Alternative approaches have been described: a distal ileostomy with end-to-side ileal anastomosis (Bishop-Koop), known as a "distal chimney enterostomy". This procedure consists of a Roux-en-Y anastomosis between the end of the proximal segment and the side of the distal segment at least 3 to 5 cm from the open end. The open limb of the distal segment is used as an ileostomy. A variation of this technique requires an angulating proximal segment that is obliquely anastomosed with the distal stump.

Proximal chimney enterostomy, the so-called Santulli procedure, consists of a proximal ileostomy with end-to-side ileal anastomosis. The end of the distal limb is anastomosed to the side of the proximal limb, the end of which is used as the enterostomy. This technique should facilitate irrigation as well as decompression of the proximal small bowel. The enterostomies can be closed by an end-to-end anastomosis when through passage of the intestinal contents is established, usually after 7–12 days [14].

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93

Hirschsprung's Disease

Vincenzo Jasonni and Alessio Pini Prato

93.1 Introduction

Although sporadic reports of patients with suggestive clinical features date back to the seventeenth century, the first telling and concise description of the disease is that of Sir Harald Hirschsprung, a Danish pediatrician who reported the details of his series of two patients at the Society of Pediatrics in Berlin in 1886.

Hirschsprung's disease (HSCR) is one of the most common causes of intestinal obstruction in the newborn. The disease occurs as a consequence of abnormal migration/differentiation of neural crest derived neuroblasts into the developing gut that determines absence of intestinal intramural ganglia. Synonyms of HSCR are Congenital Megacolon and Intestinal Aganglionosis that indicate severe bowel loops distension and absence of intramural ganglia, respectively. Most patients present in infancy and early diagnosis is important to avoid complications. Overall prognosis is good. In fact, with proper treatment most patients live normal adult lives [1].

93.2 Etiology and Pathogenesis

The disease is caused by the failure of neural crest derived neuroblasts to migrate cranio-caudally during weeks 5–12 of gestation. This abnormal migration determines congenital absence of intestinal intramural ganglia within the enteric nervous system (myenteric and submucosal plexuses) with variable distal bowel involvement. This intrinsic innervation is named "gut mini brain" or enteric nervous system (ENS) and coordinates movements, immune functions, and secretion of the gut. Subsequently its abnormality determines functional bowel obstruction and facilitates the onset of severe infections known as Hirschsprung associated enterocolitis (HAEC), which represent the most severe complication of the disease.

The diseased bowel begins at the pectinate line (anal canal shows physiologic aganglionosis) and extends proximally with variable distal bowel involvement. Aganglionosis extending up to the splenic flexure of the colon is named classic HSCR (most frequent, accounts for up to 80% of cases), that extending beyond (confined within the large bowel) is named long HSCR (10%), whereas that involving the whole large bowel (usually extending to at least 5–7 cm of terminal ileum) is defined Total Colonic Aganglionosis (TCSA) or ultralong HSCR (10%). Total bowel involvement is extremely rare and is nearly incompatible with life [1–3].

This is a complex genetic disease as suggested by the high proportion of sporadic cases, variable expressivity, incomplete sex-dependent penetrance, and variable risk of sibling recurrence. In 1994, mutations affecting the RET proto-oncogene on chromosome 10q11.2 were identified in HSCR patients, the same being also involved in different diseases such as multiple endocrine neoplasia type 2A and 2B and sporadic and familial medullary thyroid carcinoma. Since then, loss-of-function of RET tyrosine-kinase has been demonstrated in approximately 50% of familial and 10–15% of sporadic HSCR cases [4–6]. Eight other HSCR susceptibility genes have been identified to date namely GDNF, EDN3, EDNRB, NRTN, ECE1, PHOX2B, SOX10, and ZFHX1B. Amongst these, GDNF belongs to the Ret signaling pathway.

Strong genetic background is also confirmed by the frequent presence of associated anomalies that can basically involve all systems and organs. Association can be either syndromic or not. Down syndrome (trisomy 21) is the most common chromosomal abnormality associated with the disease, accounting for approximately 10 percent of patients. Other syndromic associations are those with congenital central hypoventilation (Ondine's course), Waardenburg-Shah, Bardet-Biedl, Mowat-Wilson, Goldberg-Shprintzen, and others. Non-syndromic association are those involving neurologic, cardiovascular, urogenital, gastrointestinal, and skeletal

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

systems, metabolism or pigmentation. Anomalies that have been encountered in HSCR patients include congenital deafness, hydrocephalus, bladder diverticulum, Meckel's diverticulum, imperforate anus, ventricular septal defect, renal agenesis, cryptorchidism, and neuroblastomas. Recent evidence suggested that the incidence of associated anomalies is somehow underestimated with true isolated HSCR being relatively rare [7–9].

93.3 Clinical Aspects

HSCR is a congenital disease, which can be familial or sporadic. It occurs in roughly 1 in 5000 live births but its incidence varies significantly among ethnic groups (1.5, 2.1, and 2.8 per 10,000 live births in Caucasians, African-Americans, and Asians, respectively). There is a strong male preponderance, with a male to female ratio of roughly 4:1. Familial cases are not infrequent. Approximately 3–5% of male siblings and 1% of female siblings of children with classic HSCR also have the disease. However, the risk is substantially higher (12.4–33%) in siblings of children with TCSA.

Symptoms range from neonatal intestinal obstruction to chronic progressive constipation in older children (Table 93.1). Although meconium delay has long been considered a characteristic feature of HSCR patients, in a recent review of more than one hundred patients, we demonstrated that "only" 64% percent of infants with Hirschsprung's disease fail to pass meconium in the first 24 hours of life [10]. More than 90% of HSCR patients had symptom onset before one year of age with difficult bowel movements, poor feeding, and progressive abdominal distention. Explosive bowel movements caused by functional colonic obstruction and hyperperistalsis are common in infants with Hirschsprung's disease. In fact, rectal examination may demonstrate a tight anal sphincter and explosive discharge of stool and gas. A small percentage of patients may not have symptoms until later in life. Common symptoms in older children include chronic progressive constipation, recurrent fecal impaction, failure to thrive, and malnutrition. One third of patients with Hirschsprung's disease present with the so-called Hirschsprung associated enterocolitis (HAEC) rather than constipation. This severe and frightening complication is characterized by explosive smelly diarrhea, abdominal distension and signs of impending septic or hypovolemic shock. HAEC still carries a significant morbidity (need for enterostomy) and up to 10% mortality [11–14].

Though clinical features do not strictly correlate to length of aganglionosis, long HSCR and TCSA tend to have worse clinical features and outcome. Total bowel aganglionosis is extremely rare and is nearly incompatible with life [14, 15].

Based on typical clinical findings, HSCR can be suspected if the child is younger than 12 months at onset, complains of meconium delay, fails to thrive, does not experience overflow incontinence or soiling, and has a tight anal sphincter with empty rectum at physical examination. Symptoms may recur after previously resolving with enemas, laxatives, or feeding changes. Conditions that can mimic HSCR in the neonatal period include cystic fibrosis, meconium plug syndrome, small left colon syndrome, hindgut atresia, anorectal malformations, ENS immaturity of the preterm, hypothyroidism, Intestinal Neuronal Dysplasia (IND) and chronic intestinal pseudo-obstruction [11, 16].

Table 93.1 Clinical features of patients with Hirschsprung's disease and incidence of symptoms

Symptoms of Hirschsprung's disease (series of 112 patients) [10]	Incidence (%)
Newborns and infants	
Abdominal distension	94
Difficult bowel movements	92
Failure to pass meconium	63
Intestinal obstruction	61
Failure to thrive	42
Enterocolitis	35
Older children	
Absence of soiling or overflow incontinence [16]	96
Chronic constipation with onset before one year of age	96
Progressive abdominal distension	85
Fecal impaction	49
Failure to thrive	27

93.4 Diagnosis

The gold standard for the diagnosis of HSCR is rectal suction biopsy (RSB) [17]. This is a safe and painless procedure that can be accomplished in an outpatient setting. The harvested specimens must be taken 1, 3 and 5 cm above the pectinate line and should contain enough submucosa to properly assess the ENS. It should be processed with histochemical staining techniques including acetylcholinesterase, alpha-naftylesterase, NADPH-diaphorase and lactate dehydrogenase for innervative assessment, and toluidine blue or hematoxylin and eosin for gross histology. The diagnostic features include absence of ganglia and thick parasympathetic nerve trunks in the submucosa and increased achetycholinesterase activity in the lamina propria. The diagnostic accuracy of RSB is close to 100% after one month of age but must be taken with care in the newborn or preterm who can only partially express all diagnostic features. In order to improve the diagnosis of HSCR we implemented an innovative instrument to perform safe RSB at the bedside of both newborn and adult patients, which was named Solo-RBT. We reported a series of 389 biopsies performed with the instrument Solo-RBT with an incidence of complications (bleeding, perforation) lower than 1% [18, 20]. Imaging can help diagnose Hirschsprung's disease. A plain abdominal radiograph may show a dilated small bowel or proximal colon. Contrast

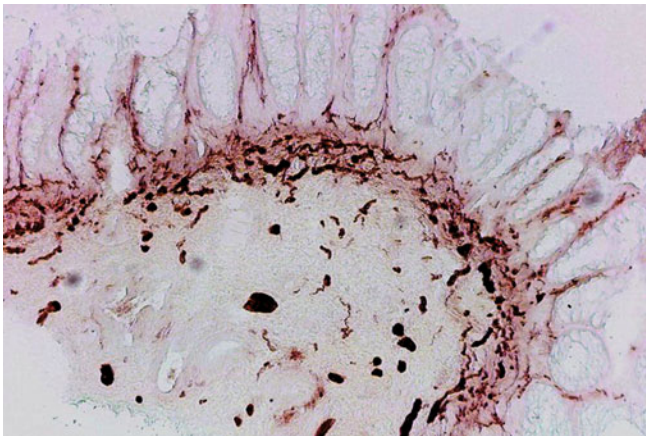

Fig. 93.1 Rectal suction biopsy of a neonatal Hirschsprung's disease. Acetylcholinesterase staining showing absence of ganglia, thick nerve trunks in the submucosal and increased acetylhcolinesterase activity in the lamina propria

enema radiographs of the colon are commonly normal for the first few months of life and indefinitely in patients with total colonic disease. After the dilation process begins, the diseased portion of the colon will appear normal and the more proximal colon will be dilated. A "transition zone" (passage from aganglionic to normoganglionic bowel) may be visible on a contrast enema radiograph. However, the aganglionic colon will extend beyond this point in about 10 percent of patients [12]. Fig. 93.1 shows contrast enema radiographs of an infant with Hirschsprung's disease. Anal manometry (balloon distention of the rectum) demonstrates the absence of internal anal sphincter relaxation upon rectal distention. Contrast enema and anal manometry are similar in sensitivity and specificity and can only be used as adjunctive diagnostic tools with the diagnosis only being confirmed by histochemistry with a RSB (Fig. 93.2). The usefulness of barium enema relies in the possibility of predicting the length of aganglionosis and therefore plan surgical treatment accordingly.

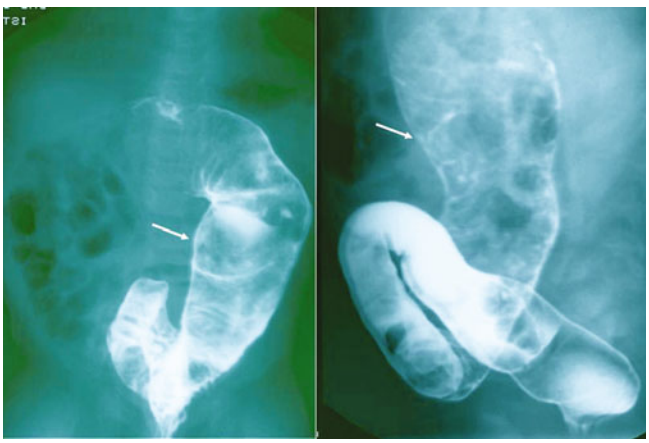

Fig. 93.2 Barium enema of a 2 month old baby showing dilated descending colon (*white arrows*). These slides do not clarify the site extent of aganglionosis and the site of normoganglionic colon

93.5 Treatment

After HSCR is diagnosed, surgery is mandatory. Serial rectal irrigation helps decompress the bowel and prevent enterocolitis in the preoperative period. This treatment is essentially a temporary non-invasive colostomy to decompress the dilated colon. In otherwise healthy newborns with undistended colons and classic HSCR, radical surgery can be attempted primarily. If the child has HAEC, TCSA, or a significantly dilated colon, a decompressive enterostomy (either a colostomy or a ileostomy) can be placed for several months while the child recovers. There are several alternative surgical procedures to treat HSCR. All work well provided certain considerations are taken into account: 1) aganglionic bowel must be removed radically, including the rectum down to the sphincters, 2) the normoganglionic bowel must be identified with intraoperative seromuscular biopsies stained with histochemistry avoiding gross anatomical considerations and blind pull-through, 3) an experienced pathologist is required for intraoperative assessment in order to correctly identify the correct site of anastomosis as well as the possible presence of associated hypoganglionosis or intestinal neuronal dysplasia and 4) surgeons must be well experienced to achieve better results and reduce the incidence of complications. The radical resection of the aganglionic bowel can be performed either with an endorectal, retrorectal, or perirectal approach (Soave, Duhamel, or Swenson procedure, respectively) depending on the surgeon's attitude. We have operated on more than 150 HSCR patients younger than 6 months of age since 1991 and we adopted either endorectal, retrorectal or perirectal approaches with similar results [10]. Recently, minimally invasive approaches have gained popularity and HSCR treatment changed accordingly [21, 22, 23]. The most frequently employed technique is, at present, the so-called Soave-Georgeson technique, which embraces the advantages of the endorectal pull-through and those of the minimally invasive surgery (Figs. 93.3–93.5) [23].

We reviewed the results of a series of patients who underwent the Soave-Georgeson procedure and compared them with those of a matched series of patients who underwent a Soave-Boley procedure (conventional laparotomic procedure). We observed similar results (complications, functional outcome, fecal continence, etc.) but shorter hospitalization and better cosmetic scores.

Basing on these considerations, we concluded that laparoscopic assisted endorectal pull-through (Soave-Georgeson technique) is the procedure of choice both in newborns and infants with HSCR.

93.6 Prognosis

Mortality rate for HSCR is relatively low, being more frequent in the preoperative period as a result of severe HAEC occurrences. Although mortality involves mainly newborns or young children, some reports described adult deaths due to septic complications secondary to severe enterocolitis. Postoperative results of the surgical treatment of HSCR appear to be satisfactory. However, despite good overall outcome, some authors showed a higher than expected incidence of problems in the long-term follow-up. Enterocolitis, constipation, failure to thrive, fecal soiling and incontinence are the most frequent complaints regardless of the surgical technique adopted [10, 14, 21]. In particular, various reports demonstrated that soiling and incontinence have an important impact on psychosocial functioning and parental criticism, and therefore on quality of life [10, 14, 15]. Outcome of classic (rectosigmoid) HSCR can be hardly compared to that of ultralong HSCR (total colonic aganglionosis) as it is well known that the latter implies a significantly worse prognosis. Nonetheless, various reports demonstrated that overall outcome of HSCR improves with time and that this occurs during the whole childhood up to adolescence or adulthood.

We recently presented the results of a series of 112 patients with HSCR [10]. We observed a 15% incidence of complications (residual achalasia, stricture, adhesions, leakage,

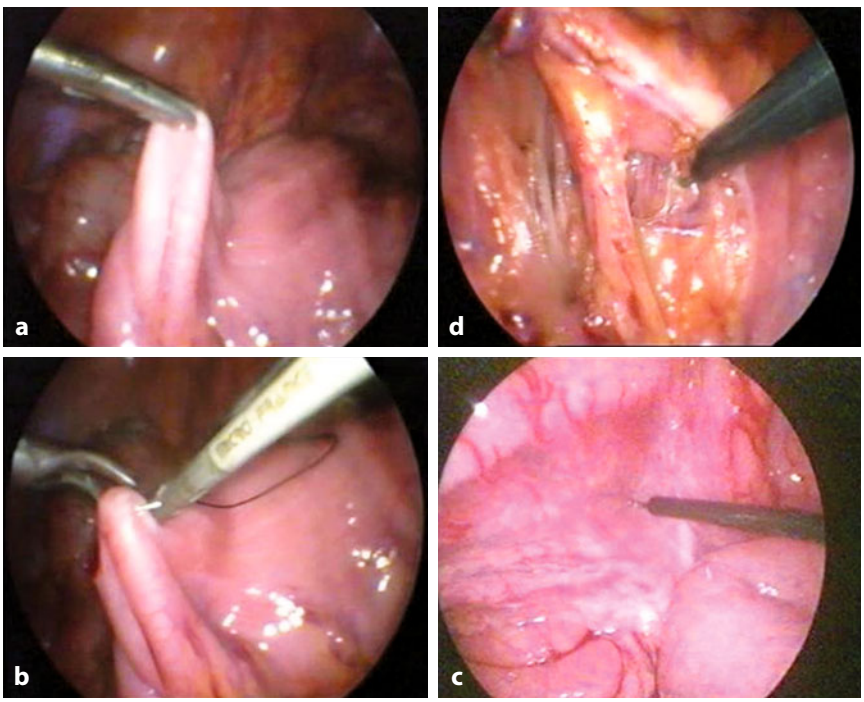

Fig. 93.3 Laparoscopic pictures of the intracorporeal steps of Soave-Georgeson technique. Anticlockwise: **a** Identification of the distended and hypertrophic colon, site of the seromuscular biopsy, **b** seromuscular biopsy to identify normoganglionic bowel, **c** commencing the preparation of the aganglionic rectum (newborn patients with virtually transparent mesentery), **d** nearly completed preparation of the mesentery before the perianal step of the procedure

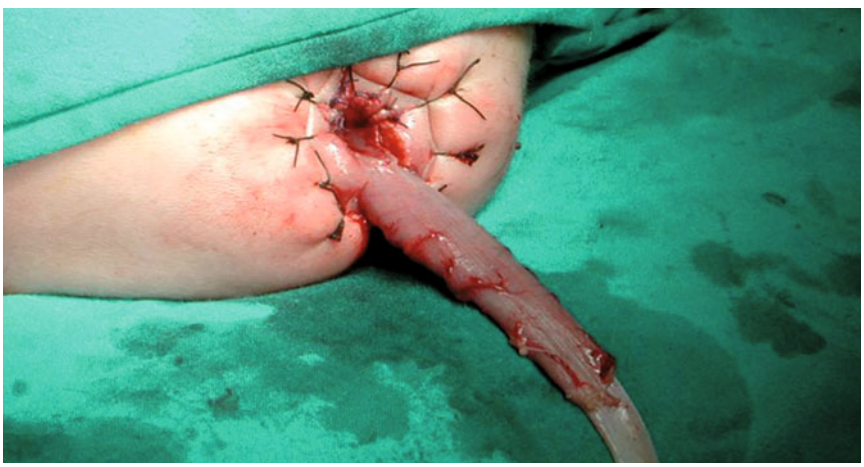

Fig. 93.4 Soave-Georgeson technique: Transanorectal pull-through of the aganglionic bowel previously prepared during the laparoscopic step

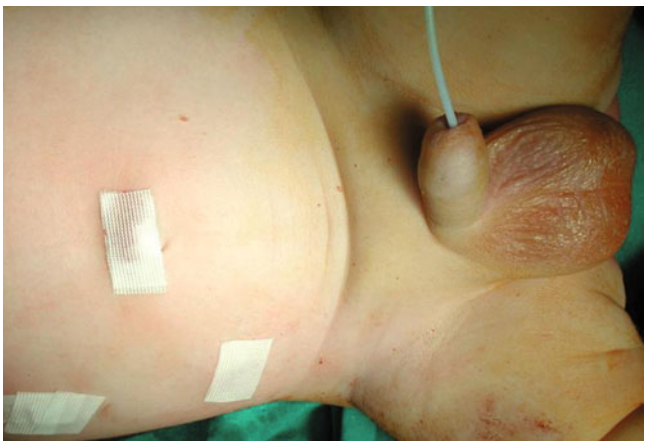

Fig. 93.5 Soave-Georgeson technique: final cosmetic appearance of the baby's belly

postoperative constipation and enterocolitis) in the classic HSCR group and roughly 60% in the TCSA group. This confirmed that outcome is significantly interfered by extent of aganglionosis. With regard to long-term outcome we observed overall good functional results. In particular, we observed 85% of excellent to good continence in the classic HSCR group of patients and 69% in the TCSA group. Psychological self-acceptance scored good to fair in 80% of classic HSCR and 55% of TCSA. Patients' perspectives were excellent to good in 98% and 100% of patients, respectively. Cosmetic results were excellent to good in 75% of classic HSCR and 18% of TCSA patients. Overall good expectations are reasonable but a number of patients with persistent issues can occur [10].

In the case of a redo operation we can expect similar long-term results and outcome provided experienced surgeons and pathologists cooperate in the radical treatment. Our series of 70 patients who underwent a redo pull-through for various reasons experienced results that do not significantly differ from those of a single pull-through. This allows optimistic expectations but suggests centralization of care in order to achieve the best for patients.

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94

Gastroenteritis and Intractable Diarrhea

Assunta Braito

94.1 Introduction

The term gastroenteritis (GE) is applied to diarrhea and vomiting caused by infectious agents involving the upper small bowel and/or colon. Diarrheal diseases are the leading cause of morbidity and mortality in infants and children under 5 years. Ten million children under 5 years die each year worldwide and 19% of these deaths are attributable to infectious diarrhea. There are an estimated 25 million neonates with gastroenteritis with a case/fatality rate of 0.6% [1].

In developed countries, a low exposure to enteropathogens and protection by breastfeeding seem to reduce the risk of newborns to acute gastroenteritis (AGE). Outbreak of the disease in hospitals is likely to be a consequence of a failure to take appropriate preventative measures.

Neonatal GE is common in the poorest areas of the world, where low levels of education, sanitation, hygiene and medical care increase the early risk of exposure to enteropathogens. Malnutrition, intrauterine growth restriction and low birth weight increase the risk of new diarrheal episodes, predisposing to chronic diarrhea, which may have a significant impact on nutritional status and on the psychomotor and cognitive development of young infants.

Recurrent or protracted diarrhea of infectious origin should be differentiated from early onset severe protracted diarrhea (SPD) or chronic diarrhea primarily due to intrinsic enterocyte defects or to immunoinflammatory enteropathies.

This chapter discusses the etiopathogenesis, transmission, clinical aspects and prevention of GE caused by those microorganisms most frequently identified as causative agents of diarrheal diseases.

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94.2 Etiology

Causative agents of GE during neonatal period include viruses, bacteria and protozoa most commonly isolated from the stools during diarrheal disease in older children and adults [2].

94.2.1 Enteric Viruses

These viruses infect the intestinal mucosa and cause GE. They should be differentiated from *Enterovirus*, which are generally associated with systemic illness. The significance of *Enterovirus* detection in the stools of infants with diarrhea is uncertain.

94.2.2 Rotavirus

Rotaviruses are by far the most common causative agents of diarrhea in children and infants worldwide. Based on common group antigens, three different *Rotavirus* groups have been identified in humans (A, B, C). Group A strains are associated with more than 95% of rotavirus infections. The subclassification into serotypes is based on epitopes VP4 and VP7, which stimulate neutralizing antibody production. Some uncommon strains seem to be endemic in neonatal units [3, 4].

Rotavirus penetrates and disrupts mature columnar enterocytes located on the brush border of the small intestine. The transfer of *Rotavirus* into the cells is probably mediated by lactase as enterocytes lacking this enzyme are not susceptible to infection.

Diarrhea results from the decrease of intestinal surface area, disruption in epithelial integrity, transient lactase deficiency and altered water and electrolyte absorption. The pathogenic role of enteric types of *adenovirus*, frequently associated with diarrhea in older children, is less common during the first weeks of life.

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94.2.3 Norovirus

Noroviruses, belonging to the *Caliciviridae*, are the leading cause of viral gastroenteritis worldwide and the commonest agent of severe pediatric AGE, especially where the antirotavirus vaccines are widely used. They are responsible for nosocomial outbreaks of diarrhea in neonatal nurseries and in adults [5, 6].

94.2.4 Bocavirus

Bocavirus, related to human *Parvovirus*, are mainly associated with acute upper respiratory infections in infants. During the course of the disease an increased rate of gastrointestinal symptoms has been observed. Its role as a causative agent of diarrhea is being investigated [7].

94.2.5 Escherichia coli

E. coli are the predominant aerobic enteric flora in humans. Coliform bacilli colonize the lower intestinal tract of the newborn during the first days of life. There are at least six pathotypes of diarrhea-producing *E. coli* with different entero- pathogenicity. Enterotoxic *E. coli* (ETEC) produces two types of toxins, one causing an immediate and reversible secretory response (ST) the other activating adenylate-cyclase (LT). The raised cAMP levels that result from stimulation of enterocytes by LT causes over-secretion of salts and water and inhibition of their absorption. ETEC has been uncommonly associated with neonatal diarrhea in areas of the world where sanitation is good. By contrast, it is frequently isolated from infants with acute watery diarrhea in less developed countries, where 16–26% of diarrheal disease in neonates is attributed to ETEC [8].

Enteroinvasive *E. coli* (EIEC) invade the intestinal epithelium causing inflammatory diarrhea. The ability to invade cells by EIEC is based on the presence of a virulence-plasmid. Little is known about its association with neonatal diarrhea.

Some strains of *E. coli* (0 55, 0111) are strongly associated with epidemic and sporadic infantile diarrhea and known as enteropathogenic *E. coli* (EPEC). EPEC are among the most important causes of bacterial diarrhea occurring during the first months of life. They have been implicated in nursery outbreaks both through vertical transmission from a contaminated birth canal and through horizontal transmission from asymptomatic adult carriers. The mechanism by which EPEC causes diarrhea involves plasmid-related pathogenicity and chromosomally encoded traits. Two virulence factors have been identified: one is the locus of enterocyte effacement, encoded by the LEE chromosomal pathogenicity locus. A second virulence plasmid factor probably mediates the aggregation of bacteria to enterocytes, facilitating mucosal colonization [9].

Enterohemorrhagic *E. coli* (EHEC) is rarely reported as a cause of neonatal diarrhea. Experimental data suggest that receptors for the Shiga toxin, produced from these strains, may not be developed at this age, decreasing the risk of EHEC infection for neonates [8].

Several outbreaks of enteroaggregative *E. coli* (EAEC) in nurseries have been described in developing countries. EAEC seems to be of increasing importance in industrialized countries, although rarely observed in neonates [8].

94.2.6 Salmonella spp.

Salmonella spp. is serologically divided into groups A to E according to the O antigen. More than 2000 serotypes have been identified as causes of human diseases; some of them are frequently associated with neonatal and infantile diarrhea.

The invasiveness of *Salmonella* strains is related to certain serotypes: *S. typhi*, *S. paratyphi A* and *B*, *S. typhimurium*, *S. choleraesuis*, *S. enteritidis* and *S. dublin* are particularly invasive. The invasiveness may be mediated by invasion-plasmid, a mechanism similar to *E. coli* and *Shigellae*.

Laboratory investigations have shown several strain-related differences in inducing secretory diarrhea, in invading intestinal mucosa and submucosa and in generating disseminated infection. Invasion of the submucosa is probably favoured by the production of citotoxins which penetrate and damage the epithelial cells [2].

Neonatal infection by *Salmonella* spp. is relatively common (75 cases per 100,000 infants during the first month of life). Most infections are acquired in nurseries [8].

94.2.7 Campylobacter

Campylobacter is increasingly recognized as a cause of diarrhea. *Campylobacter fetus* causes prenatal and neonatal infections, resulting in abortion, stillbirth, prematurity and systemic disease [8].

94.2.8 Shigella

Although the peak incidence of *Shigella* disease occurs during the first four years of life, symptomatic infection is rarely seen during the newborn period. The relative resistance of newborns to *Shigella* infection is not known.

94.2.9 Other Microorganisms

Other microorganisms, as *Clostridium difficile*, *Vibrio cholerae*, *Yersinia enterocolitica* and *Aeromonas hydrophila* have sometimes been associated with AGE in the newborn. Their significance as a cause of diarrhea in nurseries has not been systematically investigated.

94.2.10 Parasites

Intestinal parasites as *Giardia intestinalis*, *Entameba hystolitica* and *Cryptosporidium parvum* are rare causes of neonatal diarrhea. The occurrence of the infection is associated with high endemicity, inadequate delivery care and poor hygienic conditions.

94.3 Transmission

Microbial agents responsible for GE are almost exclusively transmitted by the fecal-oral route. The human intestine is the main reservoir of infection, although some species of *Salmonella* and *Campylobacter* are well adapted to some animal hosts. Food and water contaminated with enteropathogenic microorganisms are the commonest source of infection.

Some bacterial species (*Salmonella*, *Shigella*, EPEC), which require a low inoculum dose to cause diarrhea, can also spread from person to person or through contaminated objects. Contact with an infected person or a contaminated source is the most important means of transmission during the neonatal period. Human and formula milk have been implicated in transmission of *Salmonella* spp. to newborns [8].

Two different epidemiological patterns are described, depending on the place of delivery. In developing countries, where most infants are born at home, household contacts and overcrowding communities are the main source of AGE in the newborns. Poor hygiene and sanitation are responsible for a high attack rate and high mortality [10].

Infants born in hospital are at risk of nosocomial infection. In the nurseries, the prevalence increases directly with the length of hospital stay. Newborn babies can acquire bacterial diarrhea during the first days of life from their mother's birth canal through fecal contamination. Infection may then spread from the infected newborn to others as a result of inadequate hygiene by personnel. Other common sources of infection have been identified in fomites such as rectal thermometers, soap dispensers and oropharyngeal suction devices [11].

Rotavirus infection may also be transmitted by aerosol droplets from the upper respiratory tract. Although this view has been based on isolation of the virus from respiratory secretions and a high rate of transmission in closed communities, the evidence for airborne transmission of *Rotavirus* has not been established [8].

94.4 Clinical Aspects

The clinical consequences of acute gastrointestinal tract infection range from asymptomatic infection to severe, life threatening gastroenteritis. The degree of exposure, the virulence of the strains and the microorganism load needed to cause infection correlates with outcome.

Host-related risk factors for severe disease are the relative hypochlorydria of the newborn during the first days of life, prematurity, low birth weight, malnutrition, underlying diseases, congenital anomalies and previous gastrointestinal infections.

Colonization without illness is probably the most common outcome of infection in the newborn. It can only be detected by laboratory investigation of stool specimens when there is an outbreak of diarrhea in a nursery. Some newborn babies exposed to enteropathogenic microorganisms develop mild, self-limiting diarrhea.

A clinical definition of diarrhea during the neonatal period is not simple, as newborns have frequent relatively loose stools. It is possible that mild diarrheal episodes caused by enteropathogens are diagnosed as normal evacuations or vice versa. The clinical assessment should therefore include a history of the preceding bowel habit (the frequency and consistence of stools). The incubation period, usually calculated during outbreaks in nurseries where the index case can easily be identified, ranges from a few hours to several days (mean 3–4 days). The onset of diarrhea can be abrupt or insidious, characterized by remarkable variability, with watery, mucus-containing or bloody stools; gross bloody stools and hematochezia are rare in the newborn. Fever and vomit can be additional signs of infections, however they are inconstant and cannot be considered as key symptoms.

Severe diarrhea is infrequent in the neonatal period. The clinical presentation includes fever, vomiting, abdominal distension, dehydration, convulsions and lethargy. Chronic or recurrent diarrhea may occur as a consequence of functional damage to the mucosa of the small intestine induced by enteropathogens, leading to secondary intolerance to disaccharides or a combination of disaccharides and cow's milk proteins.

Colonic perforation, pseudomembranous colitis, megacolon and peritonitis are rare complications of *Shigella dysenteriae* type 1 diarrhea, more frequent in newborns than in older children and adults [8].

There are little data about the extraintestinal manifestations of viral diarrhea. The relationship between *Rotavirus* gastroenteritis, Reye syndrome and aseptic meningitis is controversial.

Some species of bacteria causing diarrhea give rise to extraintestinal manifestations. The clinical picture and frequency are reported in Table 94.1. The risk of extraintestinal complication is considerable with *Salmonella* spp. infection.

94.5 Prognosis

The outcome of uncomplicated diarrhea is mostly good. Clinical manifestations usually last less than 7 days.

Table 94.1 Extraintestinal manifestations of infectious diarrhea caused by entheropathogenic bacteria

	EPEC	*Campylobacter*	*Salmonella*	*Shigella*
Abscesses			++	
Bacteremia		++	+++	++*
Convulsions**		+	++	+++
DIC				++
HUS***	++	+		+++
Jaundice	+++	++		
Meningitis		+	+++	
Osteomyelitis			+++	
Pneumonia		+	++	
Respiratory Distress	++			
Septic Arthritis			+++	
UTI			+++	

* Due to *S. dysenteriae* or to other gut flora.
** Probably due to the production of a neurotoxin.
*** Hemolysis without uremia may be seen.
DIC disseminated intravascular coagulation, *HUS* hemolytic uremic syndrome, *UTI* urinary tract infection.

Table 94.2 Differential diagnosis of diarrhea in the newborn

Acute diarrhea	
– Hematochezia associated with intestinal ischemia and hemorrhagic disease	
– Necrotizing enterocolitis	
– Systemic infection of viral or bacterial origin	
Protracted/chronic diarrhea	
Metabolic and anatomic disorders	*Inflammatory and immunological diseases*
Post-infectious disaccharidase deficiency	Cow's protein intolerance
	Soya protein intolerance
Congenital diseases	
Adrenal hyperplasia	Necrotizing enterocolitis
Disaccharidase deficiency	Acquired immunodeficiency syndrome
Cystic fibrosis	Wiskott-Aldrich syndrome
Hartnup's disease	Thymic dysplasia
Hirschsprung's disease	
Intestinal lymphangectasia	
Acrodermatitis enteropatica	

Dehydration is the most important and frequent complication of gastrointestinal tract infection and generally reflects the severity of the disease rather than its etiology. The degree of dehydration is considerably less with *Shigella* infection than with diarrhea due to other pathogens, although the frequency of stool passage may be very high.

It is estimated that loss of water and electrolytes due to *Rotavirus* AGE causes at least 150,000 annual deaths in infants before 30 days of life, mostly in newborns who were malnourished or already infected by other enteropathogens. The regions most affected are South-East Asia and Sub-Saharan Africa.

Signs of poor prognosis include weight loss, prolonged capillary refill time, poor skin turgor, sunken eyes, reduced urine output and lethargy. Severe hypovolemic shock and renal failure are the commonest causes of death.

During the neonatal period, acute diarrhea may be the first manifestation of viral or bacterial sepsis. Babies with mild or severe acute diarrhea should be monitored carefully until the diagnosis and the clinical outcome are established.

AGE or protracted post-infectious diarrhea should be differentiated from a wide range of non-infectious diseases causing acute or protracted diarrhea in the newborn (Table 94.2).

94.6 Diagnosis

The diagnosis of AGE is based on clinical recognition, on evaluation of its severity by assessing the degree of dehydration and identification of its etiology by appropriate laboratory investigations.

Clinically determining the etiology of diarrhea can be useful to promptly start antibiotic treatment if necessary. A presumptive clinical diagnosis is required in developing countries where laboratory facilities are not available. The focus should be on the identification of invasive pathogens causing inflammatory diarrhea from those causing a non-inflammatory process (Fig. 94.1).

The definitive diagnosis of gastroenteritis depends on the identification of enteropathogenic agents in the stools [2]. Pathogenic strains of *E. coli* cannot be detected by routine culture methods as they are indistinguishable from normal intestinal flora. EPEC organisms can be identified by agglutination on slides with specific antisera. Blood cultures should be routinely obtained in neonates with suspected or confirmed *Salmonella* infection. Cerebrospinal fluid examination should be considered if the newborn appears to be severely ill or when *Salmonella* or other bacteria species are identified in the blood.

Various methods have been used for the detection of viruses in the stools. Elisa and Latex agglutination are most widely used for detecting *Rotavirus* and enteric *Adenovirus* in clinical samples. In many cases a causative agent is not identified by laboratory investigations; such cases are generally assumed to be of viral etiology.

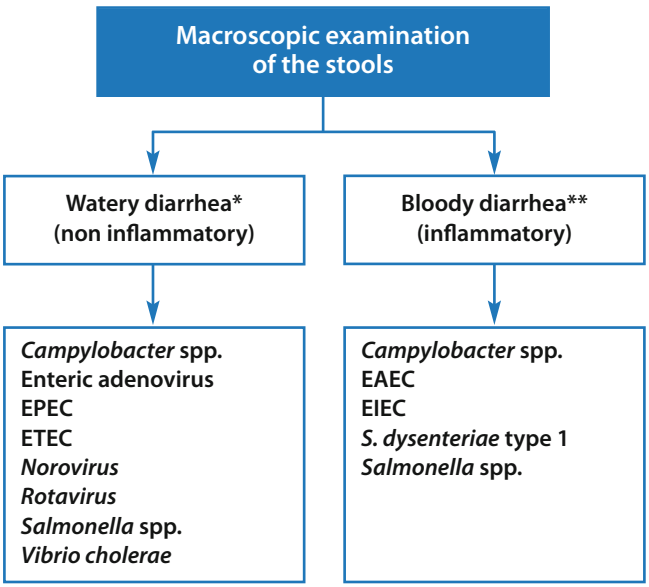

Fig. 94.1 Algorithm for clinical diagnosis of diarrhea. * Limited to small intestine tract; copious fluid stools. ** Colorectal mucosal infection. Microscopic examination for fecal leukocytes and red blood cells can be an useful indicator of inflammatory diarrhea

94.7 Treatment

The principles of management of AGE include rehydration and antimicrobial therapy if indicated. As infants are very susceptible to dehydration because of their high basal fluid and electrolyte requirements, correction of fluids and electrolytes is the mainstay of treatment of any diarrheal disease (see Chapter 95).

The degree of dehydration should be carefully assessed and rapidly corrected. Most neonates have a relatively mild disease with minimal or absent dehydration and can be treated with oral rehydration solutions (ORS). ORSs are available worldwide in several commercial forms, varying in osmolality, glucose and salt composition. Lower osmolality solutions are preferable in the newborn to decrease the stool output and shorten the duration of the disease.

Severe dehydration may require intravenous fluid replacement. Those most at risk are the youngest babies, those with profuse fluid loss with watery stools or persistent vomiting, poor urine output, and depressed level of consciousness.

Most AGE cases are self-limited and antimicrobial therapy is not routinely indicated. The use of antibiotics is confined to newborns who are severely ill or present with inflammatory diarrhea and fever due to enteroinvasive bacteria (Table 94.3). As enteropathogenic bacteria are frequently resistant to antibiotics, antibiotic sensitivity testing should be performed routinely. Although the use of antibiotics in *Salmonella* infection may induce a carrier status, antimicrobial treatment for infants under 3 months is recommended because of the substantial risk of systemic complications. Duration of therapy is between 3 and 5 days, with the exception of sepsis or meningitis when treatment should be continued at least 10 days and 2–3 weeks respectively.

Breastfeeding should be continued during an episode of AGE. Undiluted regular formula milk should be reintroduced as soon as possible, as enteral feeding during diarrhea assists recovery.

Therapy with probiotics for diarrhea in the newborn has not been proven.

Antimotility agents such as loperamide and atropine- like drugs to reduce intestinal motility should be avoided.

Racecadotril, an enkephalinase inhibitor, can reduce stool output in older children. Its effect in neonatal AGE is unknown.

Table 94.3 Antibiotic therapy in newborn diarrhea

Etiology	Antibiotic therapy
Viruses	Not indicated
E. coli	
– EAEC	Controversial*
– EIEC	Controversial*
– EPEC	Neomycin sulfate 100 mg/day**
– ETEC	Controversial**
Campylobacter spp.	Erythromycin within the first 4 days of disease
Salmonella spp.	3rd generation cephalosporins***
Shigella spp.	id

* Rarely observed in the newborn, may be useful in severely ill patients.
** Oral administration. 2nd choice drugs: colistine or polymixyne. The treatment is indicated in moderately to severely ill infants.
*** May be responsible for cholestasis.

94.8 Prevention

Currently, the best strategy for the prevention of neonatal diarrhea consists of prolonged exclusive breastfeeding.

When AGE in the newborn occurs in nursery outbreaks in developed countries, infants born to mothers with diarrhea should be isolated until the results of maternal and infant stool cultures are available. Hand washing before and after contact with each baby is the single most efficacious and least expensive measure for preventing the spread of infection from an infant or a caretaker to others [8, 11].

A wide range of strategies should be undertaken in resource-poor countries to reduce morbidity and mortality from diarrhea affecting the newborn. These include improved standards of hygiene and education, environmental sanitation, safe water supply, implementation of primary health care activities and political commitment.

Rotavirus vaccine, recommended for older children to consistently reduce the burden of AGE and its severity, is of minimal value in the neonatal period [8, 12].

94.9 Intractable Diarrhea

Severe protracted diarrhea (SPD) is a chronic condition starting soon after birth due to congenital anomalies of gastrointestinal function, leading to dependence on total parenteral nutrition (TPN). The causes of SPD are listed in Table 94.4.

SPD is a rare condition, requiring the support of specialized centers where intestinal mucosal biopsy and adequate management can be performed. Intrinsic enterocyte defects are characterized by a high degree of consanguinity in the affected families and autosomal recessive transmission has been suggested. TPN or intestinal transplantation are the only therapeutic options when ultrastructural abnormalities of enterocytes have been demonstrated.

X-linked, autoimmune enteropathy is characterized by a family history of autoimmune diseases. It is frequently associated with extraintestinal symptoms. Treatment consists of administration of immunosuppressive drugs.

Table 94.4 Causes of severe protracted diarrhea in the newborn [13–19]

Primary epithelial abnormalities
Transport and absorption systems
Absorption of carbohydrates, proteins and fats
Reabsorption of bile acids
Electrolyte secretion and absorption
Primary enterocyte abnormalities
Microvillous inclusion disease
Tufting enteropathy
Congenital deficiency of α6β4 integrin
Kindler syndrome
Primary immunological abnormalities
Autoimmune enteropathy
Severe congenital immunodeficiencies
Neutrophil granule deficiency

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95

Rehydration after Diarrhea

Carlo Bellieni

95.1 Introduction

Babies with diarrhea undergo huge fluid loss; the main problems a physician faces are choosing the fluids for rehydration and deciding on the best way to administer them. Fluids should be rapidly absorbed from the intestinal lumen: this effect is maximized with a composition of salts and other components. The combined use of an oral rehydrating solution (ORS) and an appropriate regimen of refeeding is called oral rehydrating treatment (ORT). Fluid absorption can be promoted by the enteral administration of properly designed fluids, even in the presence of ongoing losses. Water passively follows the osmotic gradient generated by the transcellular transport of electrolytes and nutrients. This is why water alone cannot be usefully employed for oral rehydration. Nevertheless, with appropriate ORS, most dehydrated children can be rehydrated successfully without resorting to parenteral intravenous (or intraosseous, when necessary) therapy; here we will see when and how, and in which cases intravenous (iv) rehydration is suggested.

95.2 Mechanism of Enteral Rehydration

The main mechanism by which ORS is effective is the coupled transport of sodium and glucose molecules at the intestinal brush border. This process is known as co-transport: the absorption of a molecule of an organic substrate promotes the absorption of an ion of sodium from the small intestine which, in turn, provokes the rapid absorption of a molecule of water. Co-transport across the luminal membrane is facilitated by the protein sodium glucose co-transporter 1 (SGLT1); once in the enterocyte, the transport of glucose into the blood is facilitated by glucose transporter type 2 (GLUT2) in the basolateral membrane, through the gradient promoted by the Na^+/K^+ ATPase [1]. This mechanism remains intact, even in patients with severe diarrhea [2]. That is why an ORS should basically contain water, sodium and glucose or another co-transporter, in well-determined proportions.

95.3 Types of Enteral Rehydration Solutions

Glucose is the most used co-transporter, though different types of co-transporters of sodium (e.g., aminoacids or cereals) have been proposed [3, 4]. The composition of some ORS is detailed in Table 95.1. A high concentration of co-transporters increases the osmolarity of the solutions, and may decrease rather than improve sodium and water transport into the bloodstream [5]. Solutions of lower osmolarity, but that maintain the 1:1 or 2:1 glucose to sodium ratio [6], perform optimally as oral solutions for diarrhea management.

ORS often contain 20 mEq/L of potassium and 10–30 mEq/L of base in the form of citrate or bicarbonate (Table 95.1) [7]. In May 2004, the WHO and the United Nations Children's Fund (UNICEF) released a joint statement to decrease diarrhea deaths among the world's most vulnerable children [8]. This statement recommended two simple and inexpensive changes from previous formulations for ORS: the switch to a new lower osmolarity formulation that reduces the need for intravenous fluids and shortens the duration of the episode [9]; and the introduction of zinc supplementation for 10–14 days, to decrease the duration and severity of the episode and the likelihood of subsequent infections in the 2–3 months following treatment [10, 11]. It is estimated that more than three quarters of all diarrhea deaths could be prevented with full coverage and utilization of zinc and ORS [12, 13]. Despite the evidence of benefit, there has been little progress on the widespread introduction of low osmolarity ORS and zinc for diarrhea treatment [13].

The WHO originally developed an ORS solution composed of glucose 111, sodium 90, potassium 20, chloride 80

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 95.1 Composition of WHO ORS and of some ORS products [17, 19, 20]

Solution	Osmolarity osm/L	Glucose mmol/L	Sodium mmol/L	Chloride mmol/L	Potassium mmol/L	Base mmol/L
WHO (1975)	311	111	90	80	20	30 (bicarbonate)
ESPGAN guidelines (1977)	200-250	74-111	60	60	20	10 (citrate)
WHO (2002)	245	75	75	65	20	10 (citrate)
Dicodral (Dicofarm)	205	104	30	41	20	10 (bicarbonate)
Dicodral 60 (Dicofarm)	221	90	60	37	20	14 (citrate)
Dioralyte (Sanofi-Aventis)	240	90	60	60	20	10 (citrate)
Electrolade (Thomson & Ross)	251	111	50	40	20	30 (bicarbonate)
Pedialyte (Abbott)	250	139	45	35	20	10 (citrate)
Rapolyte (KoGEN)	250	110	60	50	20	10 (citrate)

and bicarbonate 30, all in mmol/L [7]. However, as in developed countries, viral gastroenteritis is the usual cause of diarrhea and is associated with less severe salt losses, so the sodium content of the original WHO ORS solution was considered excessive, because higher osmolarity decreased rather than increased intestinal sodium and water absorption, and hypernatremia was reported with its use [14]. In 2002, the WHO proposed a new ORS solution, composed of glucose 75, sodium 75, potassium 20, chloride 65 and citrate 10, all in mmol/L [15]; this preserves the 1:1 molar ratio of sodium/glucose that is critical for efficient co-transport of sodium. It has a reduced osmolarity (245 mOsm/L) compared with the previous formulation (311 mOsm/L).

ORS with complex carbohydrates (derived from rice or carrots) should be given to infants only when they have already been eating solid food, and therefore should not be given to infants who are less than 4 months old [16].

95.4 Assessing Dehydration to Decide the Type of Fluid Therapy

One of the main concerns when dealing with infantile diarrhea is the mode of fluid administration. To this aim, dehydration was formerly divided into mild, moderate and severe, according with the amount of fluid loss calculated through a series of clinical signs; iv fluids were reserved for the last category (dehydration > 10%). As the boundaries between the three degrees were difficult to assess, more recently only two degrees of dehydration (some dehydration and severe dehydration) have been proposed [17].

The British Guideline Development Group (GDG) proposed a new and easy clinical assessment scheme (Table 95.2) that divides patients into no clinically detectable dehydration, clinical dehydration and clinical shock [17, 18], the last group requiring specific emergency management with administration of iv fluid boluses. When the patient has been assigned to one of the three groups, an easy flow-chart helps to decide the type of rehydration needed (Fig. 95.1). The GDG classification is useful because a small infant with gastroenteritis might experience sudden severe fluid loss at the onset of gastroenteritis sufficient to cause hypovolemic shock, needing iv therapy, before any signs of dehydration (for example, dry mucous membranes or reduced skin turgor) were present. Nevertheless, some signs can be common to different classes of dehydration: both dehydration and shock might be associated with a change in conscious state, though to different extents. The GDG concluded that when there was uncertainty the safe approach would be to treat as though shock were present [17]. Red flag signs (altered responsiveness, sunken eyes, tachycardia, tachypnea, reduced skin turgor) should alert the clinician to a risk of progression to shock (Table 95.2). Children with such red flag signs require especially careful consideration and close monitoring. For instance, tachycardia (a red flag sign) would be of even greater concern if it worsened over time, pointing to a serious risk of clinical deterioration and shock.

Babies with dehydration will usually be treated with oral fluid rehydration, those with red flag symptoms and/or evidence of deterioration will require careful management, probably in a hospital setting, while those with suspected or definite shock will require emergency iv treatment in hospital (Fig. 95.1). The GDG considered that recognition of the symptoms and signs of dehydration and shock needs considerable expertise. Clinicians therefore require training and experience in order to ensure competence in assessing children with gastroenteritis.

95.5 Modes of Alimentation

Newborns who cannot drink can be offered ORS through a nasogastric tube. If babies vomit ORS persistently, continuous infusion through a nasogastric tube may improve tolerance. The GDG [17] counsels treating suspected or confirmed shock with a rapid intravenous infusion of 20 mL/kg of 0.9%

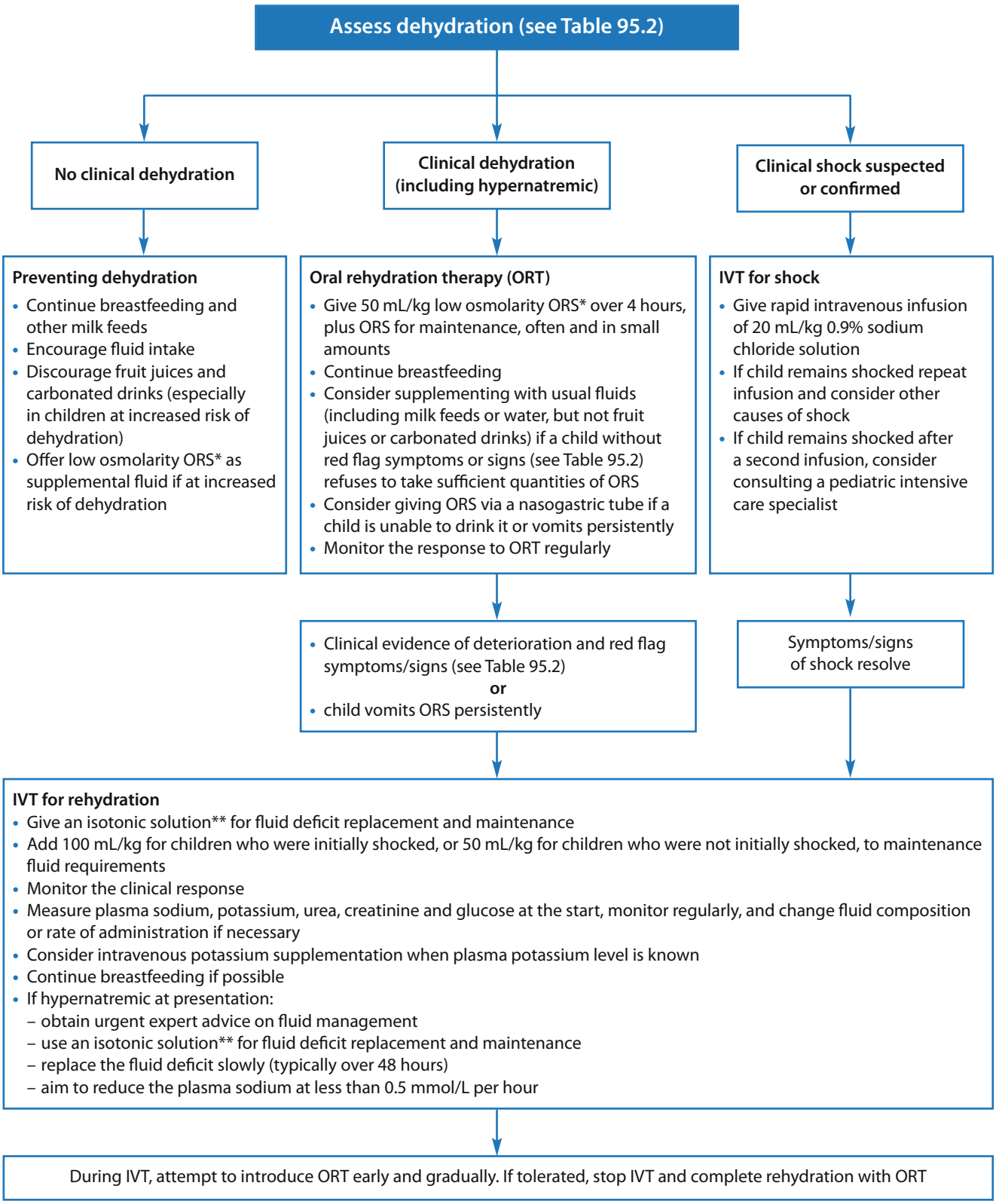
Table 95.2 Assessment of dehydrated patients. From [17]

	Increasing severity of dehydration →		
	No clinically detectable dehydration	Clinical dehydration	Clinical shock
Symptoms (remote and face-to-face assessments)	Appears well	Appears to be unwell or deteriorating	–
	Alert and responsive	Altered responsiveness (for example, irritable, lethargic)	Decreased level of consciousness
	Normal urine output	Decreased urine output	–
	Skin color unchanged	Skin color unchanged	Pale or mottled skin
	Warm extremities	Warm extremities	Cold extremities
Signs (face-to-face assessments)	Alert and responsive	Altered responsiveness (for example, irritable, lethargic)	Decreased level of consciousness
	Skin color unchanged	Skin color unchanged	Pale or mottled skin
	Warm extremities	Warm extremities	Cold extremities
	Eyes not sunken	Sunken eyes	–
	Moist mucous membranes (except after a drink)	Dry mucous membranes (except for "mouth breather")	–
	Normal heart rate	Tachycardia	Tachycardia
	Normal breathing pattern	Tachypnea	Tachypnea
	Normal peripheral pulses	Normal peripheral pulses	Weak peripheral pulses
	Normal capillary refill time	Normal capillary refill time	Prolonged capillary refill time
	Normal skin turgor	Reduced skin turgor	–
	Normal blood pressure	Normal blood pressure	Hypotension (decompensated shock)

sodium chloride solution. A second rapid intravenous infusion of 20 mL/kg of 0.9% sodium chloride solution should be administered if a child remains shocked after the first infusion, and possible causes of shock other than dehydration must be considered. If the rapid intravenous infusions resolve the symptoms/signs of shock, rehydration with intravenous fluid therapy should be started. For fluid deficit replacement and maintenance (if the baby is not hypernatremic at presentation), an isotonic solution such as 0.9% sodium chloride, or 0.9% sodium chloride with 5% glucose should be used. For those who require initial rapid intravenous fluid boluses for suspected or confirmed shock, 100 mL/kg for fluid deficit replacement should be added to maintenance fluid requirements, and the clinical response should be monitored; for those who are not shocked at presentation, 50 mL/kg for fluid deficit replacement should be added to maintenance fluid requirements, and the clinical response monitored; plasma sodium, potassium, urea, creatinine and glucose at the outset should be measured, and fluid composition and rate of administration should be monitored regularly and altered. If necessary, provision of intravenous potassium supplementation should be considered once the plasma potassium level is known.

If intravenous fluid therapy is required in a newborn presenting with hypernatremic dehydration:

- use an isotonic solution such as 0.9% sodium chloride, or 0.9% sodium chloride with 5% glucose, for fluid deficit replacement and maintenance
- replace the fluid deficit slowly, typically over 48 hours
- monitor the plasma sodium frequently, aiming to reduce it at a rate of less than 0.5 mmol/L per hour [17].



* 240–250 mOsm/L (Table 95.1)
** Such as 0.9% sodium chloride, or 0.9% sodium chloride with 5% glucose

Fig. 95.1 Fluid management. From [17]

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96

Necrotizing Enterocolitis

Elvira Parravicini and Federica Fromm

96.1 Historical Prospective and Epidemiology

The very first time Necrotizing Enterocolitis (NEC) appeared in medical literature was 1952, when two articles published in Z. Kinderheikd described a particularly severe form of enteritis, which the authors called *Enterocolitis Ulcerosa Necroticans*. Schmid described the pathology [1] and Quaiser the clinical aspect [2] of the diseases. The year after, the same authors named this condition Necrotizing Enterocolitis (NEC) [3]. It was only in 1964 that Berdon from the New York Babies Hospital described the clinical and radiological findings of the disease [4]. Since the first description of NEC, multiple etiologic factors have been identified, however many questions related to pathogenesis, early diagnosis, treatment and prevention, remain unanswered.

Although the incidence of NEC widely varies by year, by country and by institution, prematurity and low birth weight remain the most significant risk factors. In fact, the overall incidence is 1–5 per 1000 live births, but dramatically increases to 100 per 1000 in very low birth weight (VLBW) and to 200 per 1000 in extremely low birth weight infants (ELBW). In 2008 the Vermont Oxford network reported 2.4 and 9.9% as the 25th and 75th percentiles of NEC rate respectively in VLBW infants. Typically NEC affects preterm infants at about 3–4 weeks of life and often occurs in clusters, suggesting an infectious etiology. The disease is relatively more common in black infants, but both males and females can be affected. In full term infants NEC is a rare event, occurs within the first few days of life, and is associated with pre-existing conditions such as perinatal asphyxia, congenital heart defects, and polycythemia. The mortality rate associated with NEC is highest for the smaller infants and those requiring surgery and ranges from 10–50% [5].

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96.2 Pathogenesis

The pathogenesis of NEC is multifactorial and, regardless of the triggering mechanisms, the resultant outcome is significant inflammation of the intestinal tissues, release of inflammatory mediators, and down-regulation of cellular growth factors, all of which lead to variable degrees of intestinal damage. Given that NEC mainly occurs in premature infants, its pathogenesis definitively correlates with developmental immaturity of the GI tract. Other significant risk factors include intestinal ischemia, sepsis and enteral feedings.

96.2.1 Intestinal Underdevelopment

Premature infants are at high risk for NEC because of their gastrointestinal immaturity. Although enteral feedings can enhance its maturation, motility is quite underdeveloped before the third trimester in both animal models and extremely premature infants and can be impaired in neonates who experience asphyxia [6]. Impaired digestion and absorption of nutrients can contribute to intestinal injury. An animal model of NEC was established in piglets by creating intestinal loops provoking dysmotility and by administration of intraluminal injection of acidified casein, which mimics enteral feeds [7]. Other animal data suggest that overproduction and/or accumulation of small chain fatty acids in the intestinal lumen can induce concentration-dependent injury to the intestinal mucosa [8]. Another point of weakness of the GI tract of premature infants is the immaturity of the epithelial barrier in both its structural and biochemical components. Epithelial cells lining the intestinal tract are charged with major tasks related to the protection of the mucosa. These mechanisms include the enhancement of salt and water secretion to flush pathogens and toxins, the production of intestinal mucins by goblet cells and the secretion of antimicrobial molecules by Paneth cells [9]. These defense mechanisms can be severely

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

underdeveloped in premature infants with consequent overgrowth of pathogens and increased susceptibility to injury.

96.2.2 Intestinal Ischemia

The histological findings of NEC, including hemorrhagic lesions, subserosal collection of gas and gangrenous necrosis, have suggested that ischemia contributes to the pathogenesis of NEC. It is most likely that the first event begins within the intramural microcirculation and the submucosal arteriolar plexus, as these represent the principal sites of resistance regulation in the intestin. Bowel ischemic injury can be induced by decreased perfusion secondary to hypotension, decreased cardiac output or by stealing phenomenon via a patent ductus arteriosus or by diving reflex, where blood flow is diverted from the intestine towards heart and brain. Ischemia can also be secondary to mucosal surface inflammation, leading to villous arterioles and submucosal vessel damage, which may play a critical role in disease extension. Regulation of vascular resistance in neonatal intestine is mainly determined by a balance between the endothelial production of two molecules, the vasoconstrictor peptide endothelin-1 (ET-1) and the vasodilator free radical nitric oxide (NO). Under normal conditions high blood flow is maintained as the balance favors NO-induced vasodilation. However, in the transition from fetal to newborn life, especially in premature infants, a reduced endothelial production of NO can predispose to ischemic injury. Moreover, the ET-1:NO balance can be altered with prevalence of vasoconstriction by factors that disrupt endothelial cell function, including ischemia-reperfusion, sustained low-flow perfusion, or production of proinflammatory mediators. Eventually the interaction of ET-1 and NO might facilitate rapid extension of ischemia into larger portions of the intestine [10].

Although hypoxia and ischemia contribute to NEC pathogenesis, several studies suggest a stronger association with abnormal bacterial colonization, sepsis and enteral feedings [11].

96.2.3 Sepsis

Although many infants affected by NEC have negative culture findings, several organisms have been associated with outbreaks of NEC, including *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus epidermidis*, and bacteria belonging to the *Clostridia* family. However it is unclear whether these organisms are at the origin or consequential to the event.

Premature infants are often colonized by pathogenic species, with a reduced degree of colonization by normal microbial flora such as *Bifidobacterium* and *Lactobacillus* species, which are found in healthy full term and breast-fed infants [12].

Colonization by pathogenic bacteria or a paucity of commensal bacterial flora may contribute to the pathogenesis of NEC. Rat pups colonized with *Staphylococcus aureus* and *Escherichia coli* demonstrated increased incidence and severity of NEC compared with those whose intestines were populated with various bacterial species [13]. On the contrary, it is known that animal models devoid of bacteria fail to develop intestinal inflammation [14]. Moreover, many preterm infants receive frequent exposure to broad-spectrum antibacterial agents, further altering the intestinal bacterial environment. Outbreaks of NEC have been associated with *Enterobacter sakazakii*, a known contaminant of powdered milk formula, also implicated in episodes of sepsis and meningitis [15]. The presence of this bacteria in the intestine is strongly associated with development of NEC, as demonstrated in animal models, where oral feeding contaminated with *Enterobacter sakazakii* induced NEC [16].

96.2.4 Enteral Feedings

NEC occurs mainly in infants who have been fed, although there is no evidence that delaying introduction of enteral feeds decreases the incidence of the disease. Rather it has been demonstrated that NEC occurs more often when high volumes of enteral feeds are achieved [17] and that a prolonged use of trophic feeds is protective compared with quick increments in the first 10 days of life [18]. Promotion of breastfeeding reduces the incidence of NEC by three to ten-fold [19] although there is no agreement whether donor milk has the same protection as mother's milk [20].

96.2.5 Abnormal Inflammatory Response

An abnormal inflammatory response has been advocated as the final pathway after exposure to risk factors associated with NEC, such as intestinal underdevelopment, ischemia, abnormal bacterial colonization, sepsis and formula feeding. Laboratory and human investigations suggest that these risk factors may activate the production of several pro- and anti-inflammatory cytokines in an unbalanced fashion that ultimately leads to bowel injury and necrosis. Although premature infants are capable of mounting both pro- and anti-inflammatory responses, there is evidence that the pro-inflammatory pathway is favored. Toll-like receptors (TLRs) are able to recognize molecules found on microbes and are present on various cells, especially inflammatory cells. Following activation of TLRs a complex cascade of signals occurs, culminating in the translocation of the pro-inflammatory transcription factor NF-kB (nuclear factor kappa-light-chain-enhancer of activated B-cells) to the nucleus and release of inflammatory cytokines. There is experimental evidence that intestinal epithelial cells

normally express a low level of TLRs, maintaining a status of hypo-responsiveness of intestinal epithelial cells to commensal bacteria. This process is developmentally regulated, and perturbations of this response could lead to intestinal inflammation and necrotic injury [21].

In animal models it has been demonstrated that in mother fed animals, there is a decrease in TLR4 expression in intestinal epithelium, while in formula-fed and asphyxia-stressed animals, TLR4 expression increases [22]. Moreover, it has recently been shown that TLR4 wild-type mice exposed to hypoxia and fed with formula have increased incidence of NEC when compared with TLR4 mutant mice. There is a suggestion that the increase in NEC is secondary to increased apoptosis and decreased capacity for intestinal repair [23]. Alterations in downstream signaling after TLR activation may also influence the balance of the inflammatory response. The most likely molecules involved include Tumor Necrosis Factor-Alpha (TNF-α), Platelet Activating Factor (PAF), interleukins (IL-1, IL-6, IL-8, IL-10, IL-12 and IL-18), nitric oxide and oxygen free radicals, as documented by studies on animal models and newborns affected with NEC.

Intestinal inducible nitric oxide synthase (iNOS) mRNA expression in animals exposed to hypoxia and/or formula feeding was increased when compared with the expression of maternally fed pups [24]. A study on human newborns compared the presence of iNOS mRNA by immunofluorescence in pathology specimens of infants who underwent intestinal surgical resections. All the specimens from a group of 15 infants affected with NEC were positive. On the contrary, only 2 out of 6 pathology specimens obtained from infants who underwent surgery for indications other than NEC, were positive for iNOS expression [25]. Data on a population of infants affected with NEC showed that both pro-inflammatory IL-8 and anti-inflammatory IL-1 and IL-10 were elevated in the most severely affected infants compared with those less severely affected [26]. Although these data demonstrate that both systemic anti-inflammatory and pro-inflammatory signaling is active in neonates, premature infants can be vulnerable at this level because of inappropriate regulation of their inflammatory response.

96.2.6 NEC and Blood Transfusion

There have been anedoctal reports linking cases of NEC to blood transfusions. McGrady et al observed an odds ratio for developing NEC of 15.5, during an outbreak of NEC, if a baby had received at least one blood transfusion. However, neither temporal association, nor pathophysiological mechanisms were identified [27]. Mally et al published the results of a retrospective chart review of a population of 908 premature infants who received 751 PRBC transfusions. They identified a sub group of 7 stable premature infants who developed severe NEC following elective blood transfusion for anemia of prematurity [28]. The invoked pathophysiologic mechanisms include regional alterations in oxygen delivery caused by the altered oxygen carrying characteristics of stored blood, or by transient polycythemia and hyperviscosity with local variation in blood flow in the mesenteric circulation [29]. Blood flow variation can cause endothelial dysfunction producing ischemia via nitric oxide production [30]. Finally, transfusion of blood products may cause reactions, as there are reports of Thomsen-Friedenreich cryptantigen activation (TCA), which exposes neonates with NEC to the risk of hemolysis after transfusion of blood products [31].

Although neonatal exchange transfusion [32] and intrauterine transfusion [33] have been shown to be associated with an increased incidence of NEC, there is no current evidence for an association of NEC with blood transfusion.

96.3 Clinical Presentation

NEC presents with signs of gastrointestinal dysfunction such as bilious gastric residuals, emesis, abdominal distension, hematochezia and/or signs of systemic illness characterized by apnea and respiratory distress, lethargy, temperature instability, hypotension and poor perfusion. Very often early signs are not specific, as feeding intolerance is extremely common in premature infants.

The clinical progression can vary from a slowly evolving and relatively benign course to a sudden fulminant onset rapidly progressing to massive intestinal necrosis. The most severe cases are often complicated with perforation and peritonitis, and their clinical course is characterized by cardio-respiratory compromise, metabolic acidosis, shock and multi-organ failure, requiring vigorous resuscitation and sometimes leading to death.

The age of presentation varies with gestational age, with peak occurrence within the first week of life for full term and 3rd–4th week of life for very low birth weight infants [34]. The clinical course varies as well with the infant's gestational age. A prospective observational study on a population of newborns with gestational age (GA) of 23–42 weeks identified 202 infants who developed NEC. The most common sign of NEC among infants with GA of 23–26 weeks was ileus (77%), followed by abdominal distention, emesis, pneumoperitoneum, fixed intestinal loop, gasless abdomen and bloody stools. Pneumatosis intestinalis was detected in 100% of full-term infants, but only in 29% of infants with GA 23–26 weeks ($P<0.0001$) [35].

The physical exam can be characterized by minimal findings such as mild hypotonia, little spontaneous movements, moderately distended abdomen and/or distended loops with decreased bowel sounds. In the most severe cases the infant is lethargic, unresponsive, skin is pale and poorly perfused, the abdomen can be severely distended, markedly erythematous or discolored to inspection, tender and firm to palpation.

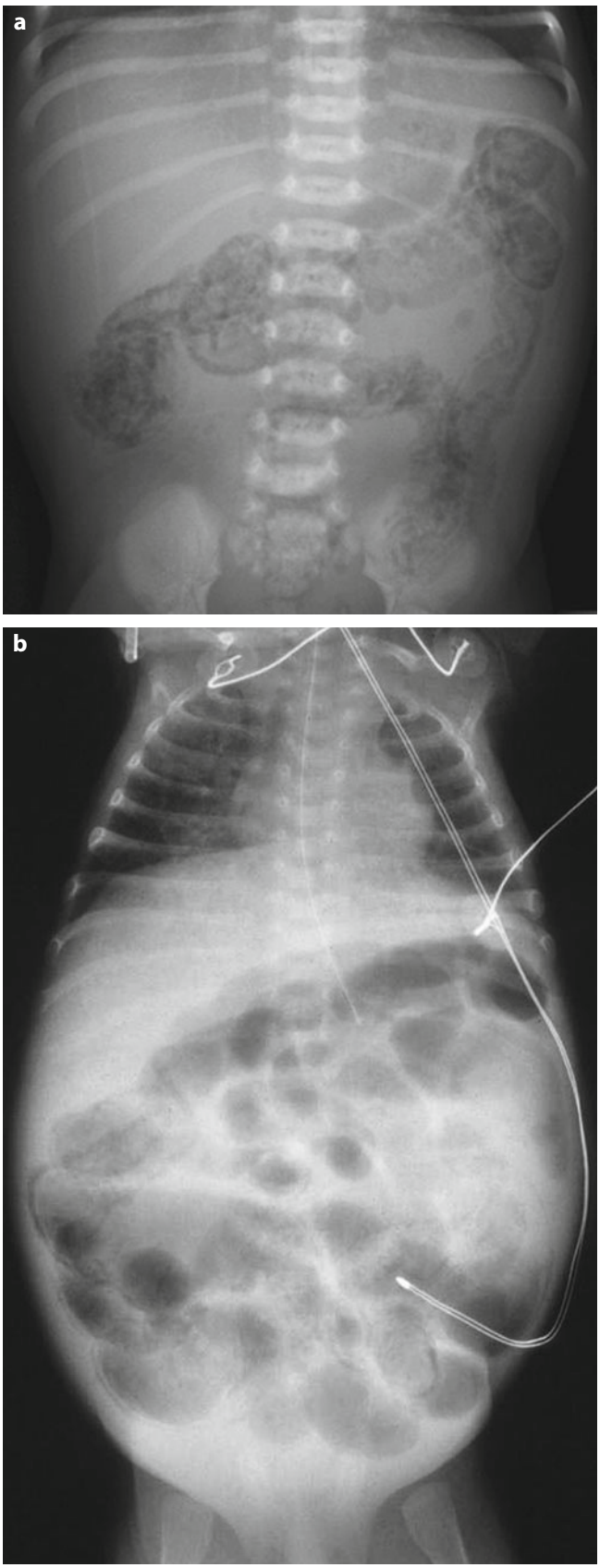

Fig. 96.1 Radiographic appearance of pneumatosis intestinalis with bubbly gas pattern (**a**) and linear gas collections in the bowel wall (**b**)

Regardless of the clinical presentation, diagnosis is established with radiologic imaging by the presence of pneumatosis intestinalis (Fig. 96.1). Other associated radiological findings include portal venous gas (Fig. 96.2), and pneumoperitoneum (Fig. 96.3).

Pneumatosis intestinalis or presence of air bubbles in the bowel wall is the result of fermentation and hydrogen production in the intestinal wall by invading bacteria, while intramural air absorbed into the mesenteric venous circulation leads to portal venous gas. Pneumoperitoneum in the absence of pneumatosis may be suggestive of spontaneous intestinal perforation, currently recognized as a disease distinct from NEC. Finally, the finding of a fixed loop that remains unchanged for 24–48 h is often associated with transmural necrosis.

Diagnostic supportive laboratory tests include a white blood cell count that can be depressed or elevated, thrombocytopenia, metabolic acidosis, electrolyte imbalance and increase of serum BUN and creatinine, markers of acute renal impairment. Culture of body fluids may grow bacteria.

A method of clinical staging was proposed by Bell in 1978 [36] and subsequently modified [37]. These criteria combine signs obtained by physical exam, signs of systemic illness and radiological findings and suggest appropriate treatment (Table 96.1).

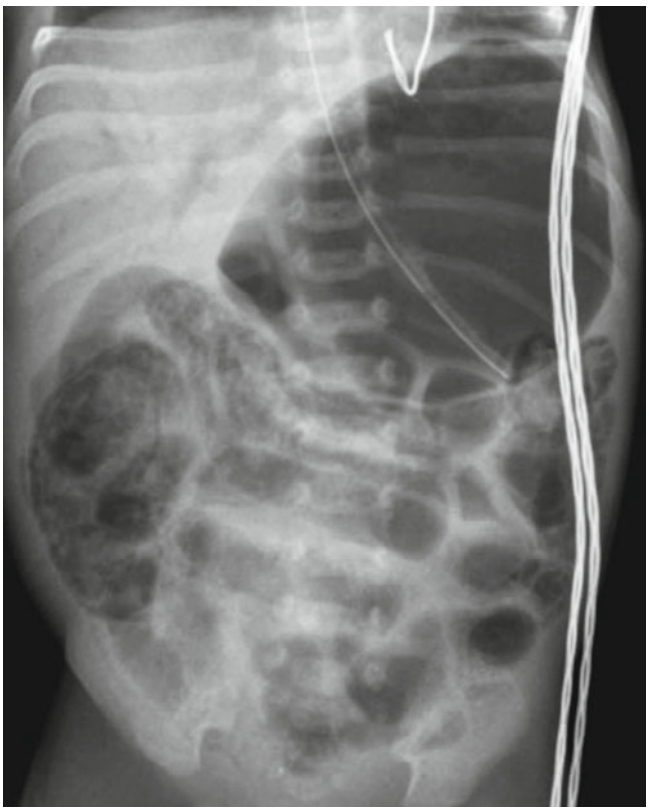

Fig. 96.2 Radiographic appearance of portal vein gas with linear dark streaks within the liver

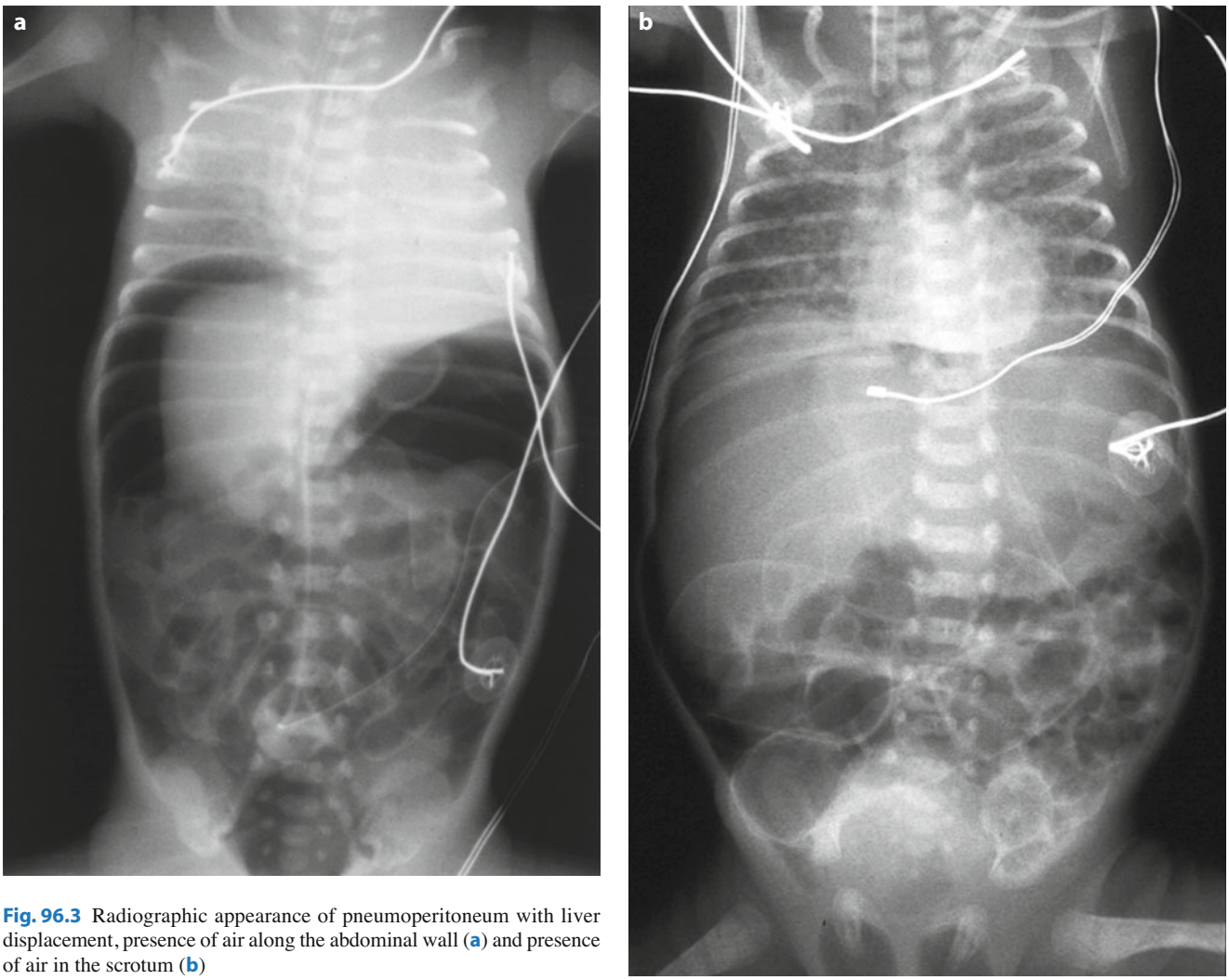

Fig. 96.3 Radiographic appearance of pneumoperitoneum with liver displacement, presence of air along the abdominal wall (**a**) and presence of air in the scrotum (**b**)

96.4 Treatment

The medical management of NEC should focus on 3 aspects: aggressive support in case of cardio-respiratory compromise, antibiotic coverage and optimal nutritional support. Intubation and ventilatory support should be initiated in infants developing apnea or respiratory compromise secondary to abdominal distension and upward displacement of the diaphragm. Hypotension is treated with fluid boluses, volume expanders and inotropic agents. Broad-spectrum antibiotic therapy should be started after body fluids have been obtained for culture. Anaerobic coverage should be added in case of bowel perforation.

Enteral nutrition is discontinued for 10–14 days and the stomach should be decompressed with an oro-gastric suctioning catheter. Nutritional support is provided via parenteral nutrition, often through a central line, provided that the infant is not bacteremic. Secondary to third space fluid losses these infants may require large volumes of fluid, especially within the first 48–72 hours.

In the acute phase of the disease radiological imaging should be repeated every 6–8 hours to assess presence of pneumatosis intestinalis and progression to pneumoperitoneum.

In case of failure of medical treatment documented by persistent metabolic acidosis, worsening respiratory distress, and shock or in presence of signs of intestinal perforation, surgery is indicated.

96.5 Prevention

Potential preventive strategies are based on prevention of the risk factors, including prematurity, infections, and hypoxic-ischemic episodes. NEC incidence can be reduced by adopting adequate feeding practices, such as early trophic feeds and preferential use of breast milk, although no conclusive study

Table 96.1 Modified Bell's staging criteria for necrotizing enterocolitis

Stage	Systemic signs	Abdominal signs	Radiographic signs	Treatment
IA Suspected	Temperature instability, apnea, bradycardia, lethargy	Gastric retention, abdominal distention, emesis, heme-positive stool	Normal or intestinal dilation, mild ileus	NPO, antibiotics × 3 days
IB Suspected	As above	Grossly bloody stool	As above	Same as IA
IIA Definite, mildly ill	As above	As above, plus absent bowel sounds with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis	NPO, antibiotics × 7 to 10 days
IIB Definite, moderately ill	As above, plus mild metabolic acidosis and thrombocytopenia	As above, plus absent bowel sounds, definite tenderness, with or without abdominal cellulitis or right lower quadrant mass	As for IIA, plus ascites	NPO, antibiotics × 14 days
IIIA Advanced, severely ill, intact bowel	As for IIB, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, DIC, and neutropenia	As above, plus signs of peritonitis, marked tenderness, and abdominal distention	As for IIA, plus ascites	NPO, antibiotics × 14 days, fluid resuscitation, inotropic support, ventilator therapy, paracentesis
IIIB Advanced, severely ill, perforated bowel	As for IIIA	As for IIIA	As above, plus pneumoperitoneum	As for IIA, plus surgery

DIC disseminated intravascular coagulation, *NPO* "nil per os" or nothing by mouth. Modified from [41], with permission by the AAP.

can clearly define the best feeding strategy. The use of a probiotics supplement has been shown as a promising preventive intervention by laboratory evidence and clinical studies.

Commensal bacteria, acquired at birth, have an important role in the maintenance of intestinal homeostasis, by inhibiting inflammatory pathways. However, premature infants are prone to pathogen colonization because of their exposure to nosocomial flora and treatments with wide spectrum antibiotics. It has been suggested that the prevalence of abnormal colonization induces a pro-inflammatory response [38]. Breast milk seems to be protective against NEC. In fact the predominance of bifidobacteria, typical of healthy individuals, is enhanced by the presence of oligofructose, a component of human milk, in the intestinal lumen. Infants who receive formula feedings without oligofructose have a predominance of clostridial organisms.

Experimental data from NEC-induced animal models show that exogenous administration of *Bifidobacterium infantis* decreases the incidence of NEC and lowers plasma levels of toxins in treated rats [39].

A recently published meta-analysis systematically reviewed several randomized clinical trials searching to demonstrate the benefit of probiotic administration in populations of premature infants. Although the risk of sepsis was not different between groups of infants treated with probiotics and controls, the risk of developing NEC and the risk of death was significantly lower in the treated population. The conclusion of this systematic review is that probiotics might reduce the risk of necrotizing enterocolitis in preterm neonates with gestational age less than 33 weeks. Several questions related to dose, duration, and type of probiotic agents still remain open [40].

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Surgical Treatment of Necrotizing Enterocolitis

Nigel J. Hall and Agostino Pierro

97.1 Introduction

Whilst many infants with NEC respond to intensive medical treatment, up to 50% develop more severe NEC requiring surgical intervention [1]. This group of infants represents a major challenge for pediatric surgeons and there is little consensus as to the most appropriate timing and nature of surgery. In addition to surgery in the acute phase, a number of infants develop late complications of NEC, either following medical or previous surgical treatment, and these complications require surgical intervention.

There is great controversy surrounding the indications for surgery in infants with NEC. Table 97.1 summarizes the indications reported in the literature. In a survey of pediatric surgeons from the United Kingdom, the indications for operation in neonates with NEC included pneumoperitoneum in 45%, clinical deterioration in 37% and intestinal obstruction in 18% [2]. The most widely accepted indication for surgery is the presence of pneumoperitoneum but this is not always demonstrable even in neonates with intestinal perforation. Other absolute indications for surgery in acute NEC include the continued deterioration of an infant that is refractory to maximal medical treatment, persisting inotrope requirement and an abdominal mass or abscess secondary to intestinal perforation.

In similarity to the lack of consensus regarding indications for surgical intervention, there is a lack of consensus concerning the ideal surgical management of infants with NEC. Whilst a number of factors may be considered in determining the type of surgical intervention including the weight and clinical status of the infant, even very low birth weight infants appear to tolerate surgery well [3]. Surgical options for the infant with NEC include primary peritoneal drainage (PPD) or laparotomy. At laparotomy, the principal surgical objectives are to control sepsis, remove gangrenous bowel and to preserve as much bowel length as possible [4–6]. Within these objectives a number of surgical options exist (Table 97.2) depending primarily on the extent of intestinal disease and the clinical stability of the infant. At laparotomy, the extent of the disease can be classified as focal when it is limited to a single intestinal segment; multifocal if it includes two or more intestinal segments with more than 50% of the small intestine viable and panintestinal when the majority of the small and large bowel are involved with less than 25% viable bowel remaining [5].

Table 97.1 Indications for surgery in infants with NEC

Absolute indications
– Pneumoperitoneum
– Clinical deterioration despite maximal medical treatment
– Abdominal mass with persistent intestinal obstruction or sepsis
– Intestinal stricture
Relative indications
– Abdominal mass
– Increased abdominal tenderness, distension and/or discoloration
– Intestinal perforation associated with persistent signs of intestinal obstruction and/or sepsis
– Portal vein gas

Table 97.2 Surgical options for advanced NEC

Primary peritoneal drainage
Laparotomy
– Resection with enterostomy
– Resection with primary anastomosis
– Proximal diverting jejunostomy
– "Clip and drop" technique

97.2 Primary Peritoneal Drainage

The use of peritoneal drainage before laparotomy, as a method of stabilizing and improving the systemic status of extremely

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 97.3 RCTs comparing laparotomy with primary peritoneal drainage in infants with perforated necrotising enterocolitis

Study	Included infants	Number	Main outcomes	Authors conclusion
Moss et al, 2006 [9]	<1500 g	PPD 55 Lap 62	No difference in mortality or dependence on PN at 90 days or length of hospital admission	No effect of procedure on outcome
Rees et al, 2008 [10]	<1000 g	PPD 35 Lap 34	No difference in survival, ventilator or PN dependence at 1 or 6 months or length of hospital admission 74% of infants undergoing PPD required delayed rescue laparotomy	Recommend early laparotomy

PPD primary peritoneal drain, *Lap* laparotomy, *PN* parenteral nutrition.

low birth weight premature infants with intestinal perforation secondary to NEC was first proposed by Marshall and Ein in the 1970s [7, 8]. Initially, it was hoped that the drainage of air and stools from an infant too unstable for a laparotomy would relieve symptoms of abdominal compartment syndrome and infection, and subsequently improve tolerance of a laparotomy. Subsequent reports, predominantly of infants weighing <1500 g, have proposed that peritoneal drainage may be used as a definitive treatment without the need for subsequent laparotomy. In fact some reports have claimed that this method of treatment may in fact be superior to laparotomy. Recently, 2 randomised controlled trials (RCTs) have addressed the issue of whether primary peritoneal drainage (PPD) or laparotomy is superior in the smallest infants with NEC [9, 10]. These are summarized in Table 97.3.

Interestingly neither trial found a significant difference in primary outcome measures between infants treated with PPD or laparotomy. However, the authors of one of the trials advocate the use of a primary laparotomy as a definitive treatment on the basis of no significant difference in outcome between the groups and a need for delayed/rescue laparotomy in 74% in infants who initially had PPD [10].

97.3 Resection of Affected Bowel and Enterostomy Formation

Resection of necrotic bowel in neonates with NEC has the theoretical advantage of reducing bacterial translocation and correcting the septic state of the patients. Following intestinal resection, the conventional view is that it is safer to exteriorize the bowel ends as the presence of peritonitis, inflammation of the bowel wall and the reduced intestinal blood supply in patients with NEC are unfavorable factors for the healing of an anastomosis [11]. Furthermore, the stoma allows adequate healing and rests the distal bowel prior to subsequent re-anastomosis [5]. There are, however, a number of potential disadvantages to this approach. It is often difficult to re-establish sufficient enteral feeding for adequate weight gain. High output stomas, in particular, carry a risk of dehydration and electrolyte imbalance, and the importance of early closure to avoid chronic salt and water loss has been highlighted. Enterostomies have also been associated with significant morbidity whilst present, including stenosis, prolapse and excoriation of the surrounding skin. Stoma closure involves a second anesthesia and is usually performed once the infant is thriving and fully recovered from the acute stage of the illness. Metabolic or physical problems, however, may demand earlier surgery. In one series, the incidence of complications in infants with enterostomies for NEC was 68% [12]. This high rate has prompted the search for alternative strategies that may avoid the need for repetitive surgery and complications associated with stomas, whilst ensuring that the underlying surgical principles of treating NEC by means of laparotomy are adhered to. Thus in many centers a primary anastomosis is preferred following intestinal resection whenever deemed safe to do so.

97.4 Resection and Primary Anastomosis

The attractiveness of primary anastomosis following intestinal resection is that a second surgical procedure can be avoided. However consideration must be given to the potential complications of intestinal anastomoses, particularly leakage and in the longer term, stricture formation. Once considered a hazardous surgical option because of the very risk of anastomotic leakage due to poor intraperitoneal healing in the presence of peritonitis, inflammation of the bowel wall and compromised intestinal blood supply, this approach has gained popularity. A number of centers have published retrospective reviews of infants treated with intestinal resection and primary anastomosis with results comparable with or in some cases favorable to those obtained with stoma formation. Furthermore, whilst resection and primary anastomosis was initially described in selected patients with focal disease and in good general condition, it is also now gaining acceptance as a valid treatment option for severe NEC, in very low birth weight infants [13] and even for multifocal disease [6].

Despite a number of reports there remains no good quality evidence to suggest whether stoma formation or primary anastomosis confers an advantage over the other approach following intestinal resection for NEC.

97.5 Operations for Panintestinal Disease

The techniques described thus far are of particular use for the infant with one or more short segments of NEC. Multiple resections and primary anastomoses may be appropriate for some infants with more widespread disease, provided careful attention is paid to the viability of resection margins. Infants with NEC affecting a large proportion of the gastrointestinal tract pose a particularly difficult problem, and treatment of this group remains particularly controversial. The surgical principles in these children are difficult if not impossible to fulfill. Due to the length of bowel involved, it is often not possible to fully remove all gangrenous intestine whilst salvaging adequate length for sustainable life. It is for these reasons that in the infant with panintestinal NEC who is unstable and critically ill, some surgeons would forego further treatment. However, when there is doubt, a number of techniques have recently been reported with the aim of allowing time for stabilization of the infants' general condition and the possibility of some healing of the gastrointestinal tract to occur. Due to the severity of the disease, the mortality with these strategies remains high.

97.5.1 Proximal Jejunostomy

Initially proposed by Martin and Neblett [14], surgical creation of a high jejunostomy in the presence of panintestinal disease has been reported in one series of 10 infants [15]. This technique allows decompression and defunctioning of the diseased intestine but does not remove gangrenous segments and may permit continued bacterial translocation. A second-look laparotomy and intestinal reconstructive surgery are performed after 6–8 weeks and the aim is once again to preserve as much bowel length as possible. This procedure may be useful in neonates with NEC affecting the majority of the intestine, but the high morbidity and mortality rate should be carefully considered.

97.5.2 "Clip and Drop" Technique

This method complies with surgical principles and also avoids stoma formation. For the infant with extensive bowel necrosis, Vaughan et al [16] advocated the resection of all segments of grossly nonviable or perforated bowel, irrigation and aspiration of peritoneal contamination, clipping the ends of remaining bowel and returning them to the abdomen. This is followed by a second-look laparotomy with delayed anastomosis 48–72 h later. In their small series, all three infants with NEC survived [16], and in a subsequent report of four infants in whom this technique was employed, one died and the remaining three required stoma formation at the second look [17].

97.6 Authors' Preferred Surgical Strategy

The authors' preferred approach to the operative management of infants with NEC is illustrated in Fig. 97.1. In infants with focal disease involving a small length of small or large bowel, a resection and primary anastomosis is usually performed, with the exception of patients unstable during the operation who are managed with a stoma at the level of the affected bowel.

In infants with multifocal disease (> 50% of the bowel assumed to be viable), various surgical options are available. Resection and one or more intestinal anastomoses (preferably not more than two) are performed when it is possible to ascertain the viability of the bowel distal to NEC without causing significant bleeding. Stoma (with or without intestinal resection),

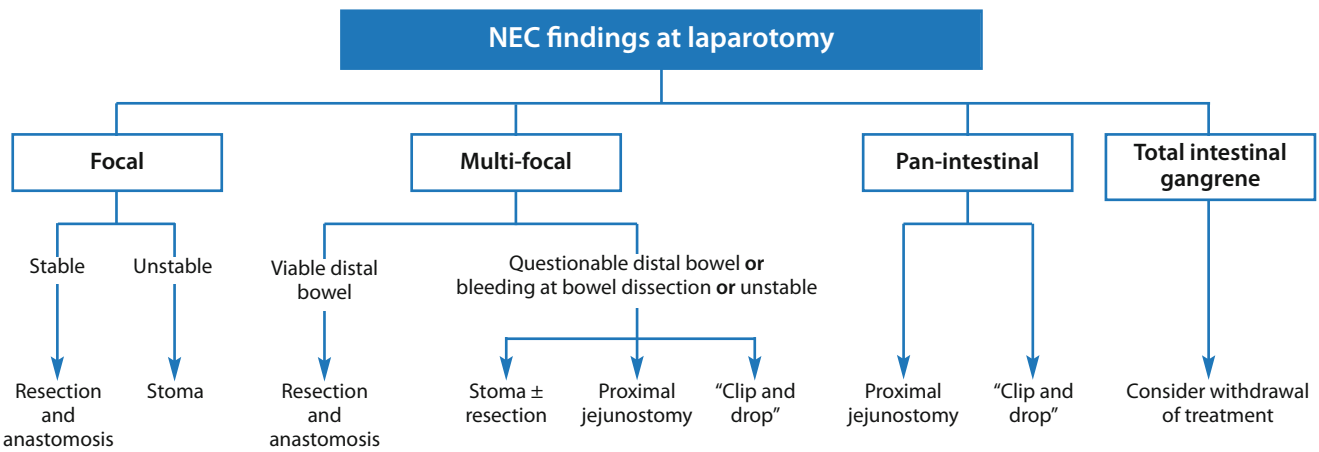

Fig. 97.1 Authors' preferred operative management of NEC. Adapted from [21]

proximal enterostomy or clip and drop technique are performed when: (a) it is not possible to ascertain the status of the bowel distal to the NEC; (b) the distal bowel is of dubious viability; (c) attempts to dissect the distal bowel cause significant bleeding and (d) the patient is unstable peri-operatively. In these circumstances, a stoma with or without intestinal resection(s) is the preferred surgical option. However, if this would lead to massive intestinal resection, the "clip and drop" technique described earlier will be adopted in an attempt to salvage as much intestinal length as possible. If the mobilization of the bowel loops affected by NEC causes significant bleeding, a high diverting jejunostomy is preferred.

In patients with panintestinal disease (>75% of small and large bowel involved), two options are considered: (a) proximal diverting jejunostomy when the intestinal resection would cause significant bleeding or loss of the majority of the small bowel and (b) "clip and drop" technique in the attempt to salvage some of the affected bowel and avoid a short bowel syndrome. In neonates with total intestinal gangrene, closure of the abdomen and treatment withdrawal are considered.

97.7 Outcome of Surgical Intervention

Central to the decision to operate on an infant with NEC and in determining the most appropriate operation to perform is the effect of any surgical intervention on outcome. Mortality from NEC is related to severity of disease, degree of prematurity and associated anomalies. In a study of 83 neonates who required a laparotomy for advanced NEC [5], the overall mortality rate was 30%. Causes of death included multisystem organ failure (n = 10), sepsis (n = 14) and congenital cardiac abnormality (n = 1). The mortality rate was higher (67%) in infants with panintestinal involvement compared with infants with focal (12%) or multifocal disease (30%). Whilst mortality is clearly the most important outcome to be considered, morbidity in survivors of NEC is often significant. In addition to the "surgical" complications related to intestinal anastomoses and stomas, other medium- and long-term complications include short bowel syndrome (affecting up to 23% of survivors of NEC) [18], hepatic cholestasis related to long-term parenteral nutrition dependency, and malabsorption. Importantly it is being increasingly recognized that infants who develop NEC are at increased risk of adverse neurodevelopmental outcome compared with preterm infants who do not develop NEC. Infants with NEC who require surgery appear to be more severely affected than those who respond to medical treatment alone [19, 20].

Clearly knowledge of these outcomes is important in counseling parents and in assessing effectiveness of treatments. As we now progress to critically determine whether a particular surgical approach is more effective than another in the setting of randomized controlled trials it is imperative that we consider these morbidities and in particular neurodevelopmental outcome in addition to mortality as part of our evaluation.

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98

Hematology and Immunology: Overview

Robert D. Christensen

98.1 The Reference Range Concept

"Normal ranges" for hematologic values of neonates are generally not available. This is because blood is not drawn on healthy normal neonates to establish such ranges, as is done with the consent of healthy adult volunteers. Instead, neonatal hematology generally utilizes "reference ranges". These ranges consist of 5th to 95th percentile values compiled from laboratory tests that were performed on neonates thought to have minimal pathology relevant to the laboratory test, or with pathology unlikely to significantly affect the test results. The premise on which the reference range concept is based is that these values approximate normal ranges, although they were admittedly obtained for a clinical reason and not from healthy volunteers.

Defining reference ranges in neonatal hematology is complicated by the fact that ranges obtained from term infants generally do not apply to preterm infants, and ranges obtained from low-birth-weight preterm infants can be very different from ranges obtained from extremely-low-birth-weight infants. As an example, a venous hematocrit of 38% would be within the reference range for a 2-hour-old neonate born at 24 weeks gestation, but would be low for a term infant.

Another problem encountered in defining reference ranges in neonatal hematology is that unstable, sick neonates might have values within the reference range, yet those values might not be optimal for patient care. As an example, for a neonate born at 24 weeks gestation, now 4 weeks old, a venous hematocrit of 24% would be within the reference range. However, if such a patient develops a nosocomial infection, becomes hypoxemic and tachycardic, and is placed on a ventilator, the hematocrit of 24% might be too low for optimal patient care. A higher hematocrit might facilitate better tissue oxygenation, diminish the heart rate, and permit better caloric utilization for growth. Therefore, just because a value falls within the reference range does not assure that it is optimal for the clinical circumstance.

Making a determination about whether a specific hematologic value is normal or abnormal often requires cognizance of the gestational age of the neonate, the age (hours after delivery) when the sample was obtained, any specific illnesses involved, the level of intensive care support given, and the anatomical site of the blood sampling (venous, arterial, capillary). Sometimes knowledge of other factors is pertinent, such as whether "stripping" of the umbilical cord was performed after delivery; and whether a capillary sample was obtained from a warmed or an unwarmed, mottled extremity. This chapter describes the reference ranges that have been assembled for use in neonatology and provides information needed to avoid pitfalls in interpreting these values. Ranges are given for erythrocytes, leukocytes, platelets, and bone marrow differential cell counts.

98.2 Erythrocytes

98.2.1 Hemoglobin Concentration

The concentration of hemoglobin in the blood and the hematocrit are among the most commonly performed of all clinical laboratory tests. The hemoglobin can be quantified by manual or automated techniques. The standard assay for hemoglobin determination, approved by the World Health Organization, is simple but highly reproducible [1]. It involves the conversion of the several forms of hemoglobin in the blood, including oxyhemoglobin, carboxyhemoglobin, and the minor quantities of other hemoglobin species present, to a single compound, hemoglobincyanide, which is then determined spectrophotometrically. The hemoglobin concentration and the hematocrit are for the most part different measurements of the same biologic variable. However, because the hemoglobin concentration is a direct measurement but the hematocrit is usually

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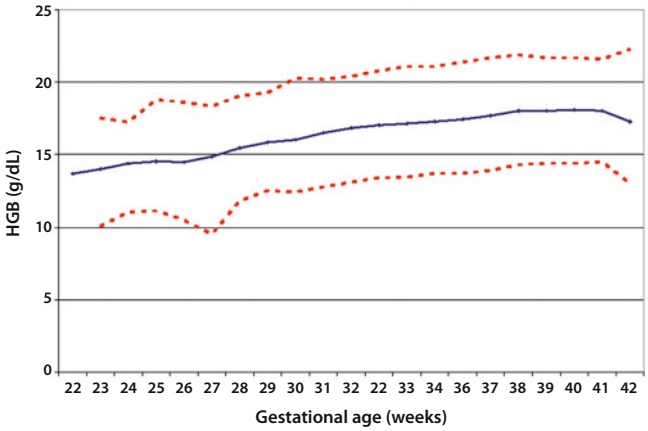

Fig. 98.1 Reference range for blood hemoglobin concentration on the day of birth (n = 24,416 patients) at 22–42 weeks' gestation. The solid line shows the mean value and the dashed lines show the 5% and 95% reference range. Reproduced from [2], with permission

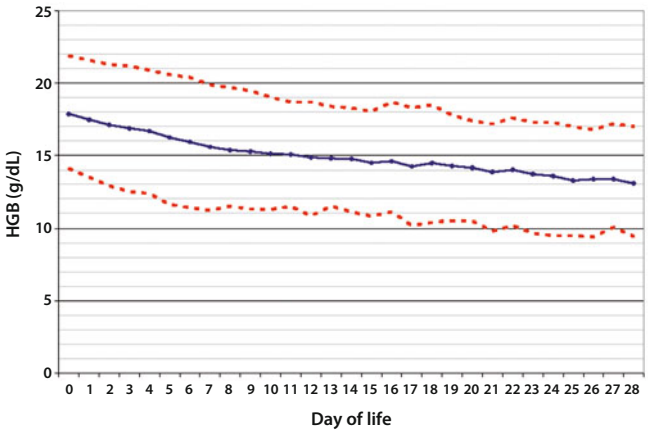

Fig. 98.2 Reference range for blood hemoglobin concentration during the 28 days after birth for neonates 35–42 weeks' gestation. The solid line shows the mean value and the dashed lines show the 5% and 95% reference range. Reproduced from [2], with permission

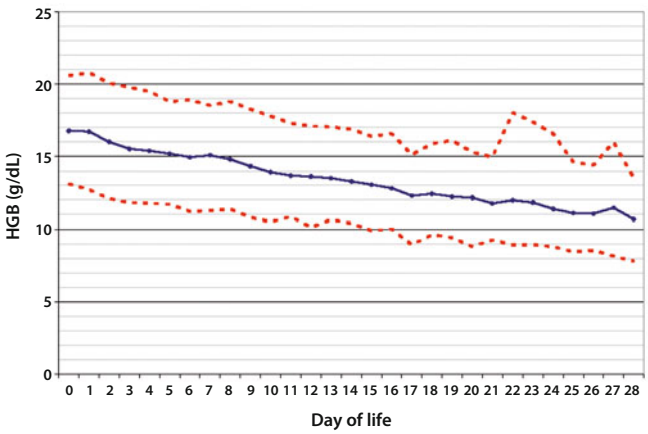

Fig. 98.3 Reference range for blood hemoglobin concentration during the 28 days after birth for neonates 29 to 34 weeks' gestation. The solid line shows the mean value and the dashed lines show the 5% and 95% reference range. Reproduced from [2], with permission

calculated, as explained later, the hemoglobin concentration has sometimes been a slightly preferred method.

Reference ranges for hemoglobin values of newborn infants 22–42 weeks' gestation [2] are shown in Fig. 98.1. In keeping with the reference range concept, values were not included in the figure if the mother had a diagnosis of placenta previa, abruptio placenta, or any antenatal hemorrhage, or if the patient had a diagnosis of hemorrhage at birth. No differences occur on the basis of gender. Postmature infants do not have higher hemoglobin concentrations than those delivered at term, unless chronic hypoxemia has occurred.

Reference ranges for hemoglobin over the first 28 days after birth are shown in Fig. 98.2 for patients 35–42 weeks' gestation at birth, and in Fig. 98.3 for patients 29–34 weeks' gestation. Hemoglobin values for patients < 29 weeks were not included in the report because virtually all had repeated phlebotomy and erythrocyte transfusions, thus confounding the slope of the hemoglobin reference range.

98.2.2 Hematocrit

In 1929, Dr. Maxwell M. Wintrobe described a clinical laboratory test that he called the volume of packed red blood cells (VPRC) [3]. The test was performed by centrifuging blood in a specifically designed glass tube called a hematocrit. Originally, "hematocrit" was the name of the tube used in the measurement, not the measurement itself. However, usage subsequently changed, and the term hematocrit now typically indicates the measurement, and the term VPRC is rarely used.

The hematocrit measurement is the proportion of the blood sample occupied by erythrocytes. The units for hematocrit are usually given as either a percentage or a decimal fraction (liters of red blood cells/liter of blood). Because neonates and young children do not have a liter of blood in their entire circulation, children's hospital laboratories sometimes choose to express the hematocrit as a percentage, not as liters per liter.

Originally, the result of the hematocrit test was read directly from the tube, after a standardized centrifugation, as the proportion of the height of the total column of blood occupied by red cells. Although an extremely simple concept, this test constituted a significant advance in clinical medicine. It was highly successful and became widely used, probably because the results were very reproducible and useful in patient care and because the test could be performed without complicated or expensive instrumentation. Subsequent modifications of the hematocrit test to permit the use of small blood samples led to its applicability to neonates. Ten years after it was described for testing adults, Waugh and colleagues reported that the hematocrit of normal infants, averaging 51.3%, was higher than that of normal adults [4].

Most hospital laboratories no longer use centrifugation methods for hematocrit determinations; instead, aperture-impedance instruments calculate the hematocrit. This is done by

electronically measuring the mean red blood cell volume and multiplying this number by the erythrocyte concentration, also measured electronically. The mean volume of the erythrocytes multiplied by their number per microliter yields the volume of red blood cells per microliter, or the hematocrit. Other types of cell counters calculate the hematocrit using laser optics, correlating the magnitude of a light pulse generated by passing a red blood cell through a laser with the cell's volume.

Many neonatologists have noticed that when manual and electronic methods for hematocrit determination are used on an individual neonate, a consistent difference occurs between the two, a difference usually not seen in testing older children and adults. Neonates tend to have a slightly higher spun hematocrit than automated hematocrit. The reason for this involves the phenomenon of trapped plasma in the spun hematocrit determinations. When blood is centrifuged in a hematocrit tube, a small amount of plasma is always "trapped" between the erythrocytes, slightly elevating the spun hematocrit value. The amount of trapped plasma is insignificant in most cases, usually in the range of 1 to 3% of the plasma volume, as determined by radioiodinated serum albumin labeling experiments [5]. However, in samples with a very high hematocrit, more plasma becomes trapped when the cells are centrifuged. Because term neonates have considerably higher hematocrits than adults, this additional plasma trapping tends to make the spun hematocrit a higher value than the automated, calculated value (which is not subject to the plasma trapping pitfall). Few spun hematocrits are used in modern neonatology, but in any NICUs doing so, this difference between spun and automated hematocrit values should be recognized.

Reference ranges for hematocrits of newborn infants 22–42 weeks' gestation [2] are shown in Fig. 98.4. As with hemoglobin values, no differences occur on the basis of gender. Reference ranges for hematocrit over the first 28 days after birth are shown in Fig. 98.5 for patients 35–42 weeks' gestation at birth, and in Fig. 98.6 for patients 29–34 weeks' gestation.

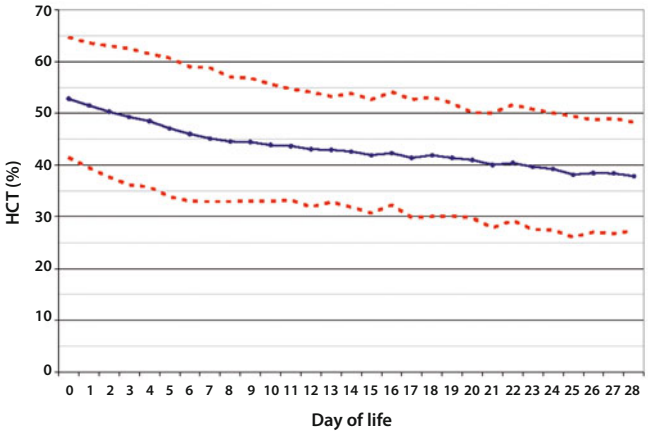

Fig. 98.5 Reference range for hematocrit during the 28 days after birth for neonates 35–42 weeks' gestation. The solid line shows the mean value and the dashed lines show the 5% and 95% reference range. Reproduced from [2], with permission

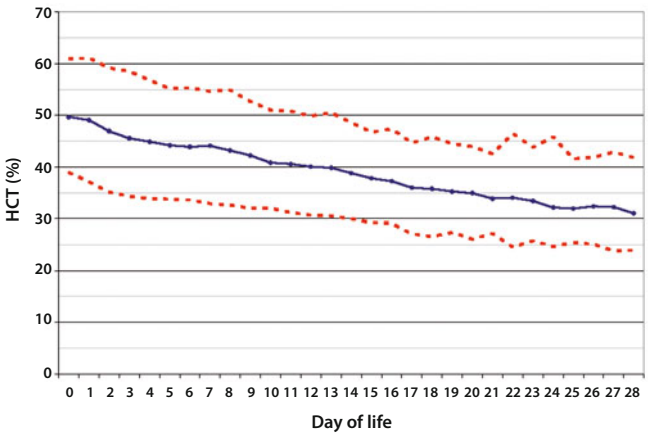

Fig. 98.6 Reference range for hematocrit during the 28 days after birth for neonates 29–34 weeks' gestation. The solid line shows the mean value and the dashed lines show the 5% and 95% reference range. Reproduced from [2], with permission

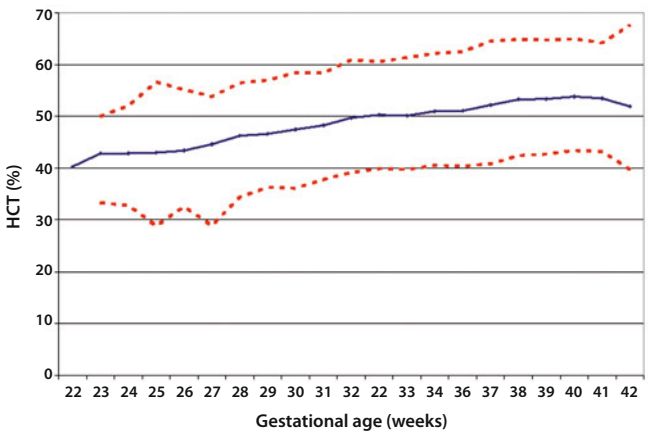

Fig. 98.4 Reference range for hematocrit on the day of birth (n = 25,464 patients) at 22–42 weeks' gestation. The solid line shows the mean value and the dashed lines show the 5% and 95% reference range. Reproduced from [2], with permission

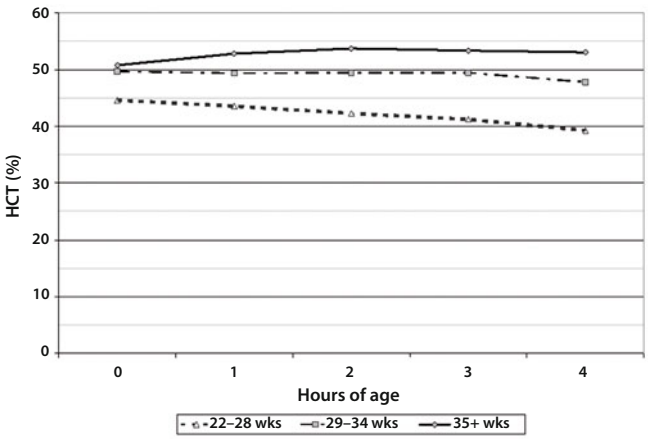

Fig. 98.7 Reference range for hematocrit over the four hours period following birth (n = 23,534 patients). Three groups are shown based on gestational age. Reproduced from [2], with permission

The hematocrit, like the hemoglobin concentration and the erythrocyte count, normally increases during the first four hours after birth. The increase, as shown in Fig. 98.7, might not occur in preterm infants. Gairdner et al [6] first reported this consistent postnatal increase in hematocrit during the hours following birth, and speculated that it was the result of intravascular concentration of blood received by placental transfusion. Its absence in preterm infants might be due to early phlebotomy losses for laboratory studies, lack of a placental transfusion (because of the desire to rapidly hand the patient to the neonatology team), or other reasons.

98.2.3 Erythrocyte Count

The erythrocyte count is the number of erythrocytes in a volume of blood, usually expressed as cells per microliter, or cells per liter. Older methods for determining the erythrocyte count used a counting chamber viewed from a microscope, but modern methods use an electronic particle counter, sampling many logs more cells than the previous methods.

98.2.4 Erythrocyte Indices

In addition to devising the hematocrit, Dr. Wintrobe introduced the concept of erythocyte indices and described methods for their calculation. Virtually every CBC includes a report of these indices. The original manual methods for making the measurements with which the erythrocyte indices were calculated have been replaced by automated instruments, which provide measurements that are more precise and reproducible. Although sometimes overlooked, these indices can provide the neonatologist with valuable information not otherwise available.

The mean corpuscular volume (MCV) is a measure of the average size of circulating erythrocytes, expressed in femtoliters (fL, 10^{-15} L). Most modern automated cell counters, using laser optics or aperture-impedance, measure the MCV of erythrocytes directly. However, as originally described, the MCV was calculated after measuring the hematocrit and the erythrocyte count by the formula:

$$\text{MCV (fL)} = \frac{\text{Hematocrit (L/L)} \times 1000}{\text{Erythrocyte count } (\times 10^{12}\text{ / L})}$$

The mean corpuscular hemoglobin (MCH) is a measure of the amount of hemoglobin in an average circulating erythrocyte. It is expressed in picograms of hemoglobin (pg, 10^{-12} gram) and is given by the formula:

$$\text{MCH (pg)} = \frac{\text{Hemoglobin (g/L)}}{\text{Erythrocyte count } (\times 10^{12}\text{ / L})}$$

The mean corpuscular hemoglobin concentration (MCHC) is a measure of the concentration of hemoglobin in an average circulating erythrocyte, and is expressed as units of grams of hemoglobin per deciliter of packed red blood cells (g/dL):

$$\text{MCHC (g/dL)} = \frac{\text{Hemoglobin (g/dL)}}{\text{Hematocrit (L / L)}}$$

Reference ranges for MCV and MCH [7] from 22–42 weeks' gestation, are shown in Fig. 98.8. A fetus at 22–24 weeks' gestation, and similarly an extremely preterm neonate at 22–24 weeks' gestation, has erythrocytes that are exceeding large, by comparison to those of adults. The MCV and MCH fall immediately when a preterm neonate receives an erythrocyte transfusion, because the MCV and MCH of the blood donor will be much lower. By 3–4 months after birth the MCV and MCH of a neonate have diminished gradually to that of the level of a normal adult (88 ± 8 fL). Thereafter, the MCV continues to decline, reaching nadir levels during the 4–6 months and then slowly increasing to adult values after the first year. When a newborn infant has an MCV of less than 94 fL, α-thalassemia trait or iron deficiency should be considered. Erythrocyte indices of Central African neonates do not differ

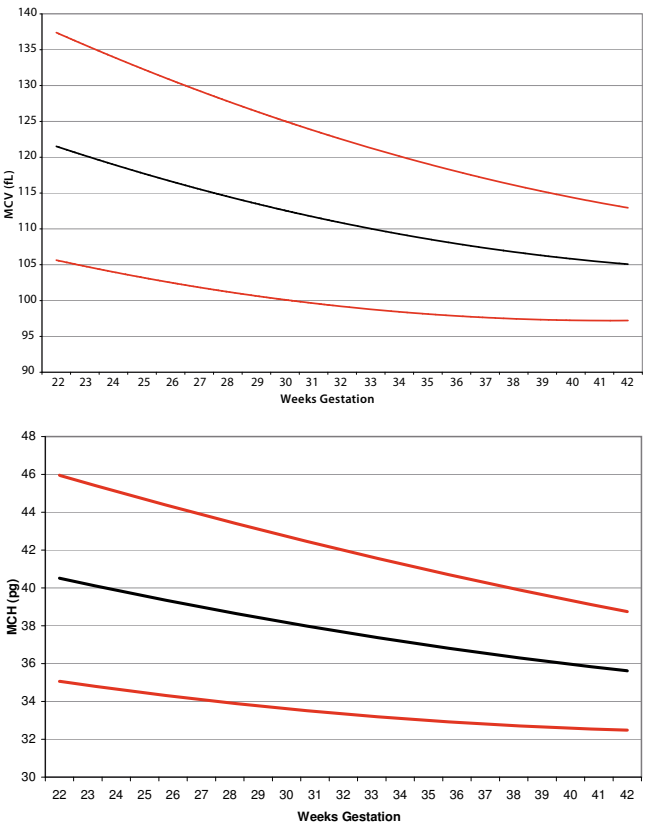

Fig. 98.8 Reference range for mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) for neonates on the first day after birth for neonates 22–42 weeks' gestation. The lower line shows the 5th percentile values, the middle line shows the mean values and the upper lines shows the 95th percentile values. Reproduced from [7]

Table 98.1 An elevated mean corpuscular hemoglobin concentration (MCHC) in a neonate can suggest the possibility of hereditary spherocytosis. Values are shown as mean and 95% confidence intervals or mean ± SD (when too few values were present to calculate confidence intervals)

	N	MCHC (g/dL)
Non-transfused neonates <12 hrs since birth	17,624	34.0 (33.0–35.1)
Neonates with Coombs (–) jaundice	1592	34.4 (33.2–35.5)
Neonates with Coombs (+) jaundice	363	34.7 (33.3–36.0)
Neonates with hereditary spherocytosis	15	36.9 ± 0.7
P Coombs (+) vs *Coombs(–) neonates*		<0.001
P Hereditary Spherocytosis vs *Coombs (–) neonates*		<0.001
P Hereditary Spherocytosis vs *Coombs (+) neonates*		<0.001

from those of neonates from other parts of the world, although the hematocrits of these neonates are somewhat lower [8].

Unlike the MCV and MCH, the MCHC does not change, either during gestation or after delivery. A reference range for MCHC of 34±1 g/dL is appropriate to all ages [6]. An high MCHC can be useful screen for hereditary spherocytosis. Michaels and associates compared automated MCHC measurements from 112 children with hereditary spherocytosis who had not undergone splenectomy with 112 matched healthy children [9] and observed that the MCHC of the group with hereditary spherocytosis was 35.9 g/dL, significantly higher than the controls (34.3 g/dL, $P < 0.001$). Similarly, newborn infants with hereditary spherocytosis have a higher MCHC than neonates with a similar peak serum bilirubin concentration who do not have hereditary spherocytosis (Table 98.1).

In addition to the erythrocyte indices, the RDW and the hemoglobin distribution width (HDW) are commonly reported on automated CBCs. The RDW is a measure of the homogeneity of red blood cell size, and the HDW is a measure of the homogeneity of red blood cell hemoglobin. The RDW is expressed as a percentage, meaning the percent of erythrocytes that fall outside (smaller or larger) the standard gated population of erythrocytes. An elevated RDW value reflects an abnormal divergence of erythrocyte size. The RDW begins to increase in cases of iron deficiency before any other change in erythrocyte indices or concentrations. The HDW is expressed in grams per deciliter (g/dL) and provides an index of divergence of hemoglobin concentration within erythrocytes. The RDW provides a numeric assessment of anisocytosis, and the HDW provides a numeric assessment of anisochromasia.

98.2.5 Reticulocyte Count

As the erythroid precursor cells clonally mature within the bone marrow their nucleus becomes pyknotic and is extruded (usually at the orthochromatic normoblast stage), and the cells are thereafter released into the blood. Cytoplasmic organelles, such as ribosomes, mitochondria, and the Golgi complex, generally persist for some time after the erythrocytes have reached the circulation. Supravital stains such as new methylene blue and brilliant cresyl blue stain the nucleic acid within these organelles (new methylene blue is a distinct chemical stain; regular methylene blue stains reticulocytes poorly). The circulating erythrocytes that contain organelles and thus have a reticulum of blue stain are termed reticulocytes [10]. Reticulocytes have generally been in the circulation for 24 hours or less and thereafter they lose the organelles and fail to stain as reticulocytes. The quantity of reticulum (i.e., nucleic acid) in an erythrocyte diminishes as it matures, thus in the youngest reticulocytes the reticulum is densely packed while in the oldest ones only a few scattered threads are found. Reticulocytes are on average about 20% larger than mature erythrocytes [11]. Scanning electron microscopy has shown that reticulocytes are not generally bilaterally indented disks like mature erythrocytes, but rather are irregularly shaped and polylobulated.

A neonatologist usually orders a reticulocyte count to assess the level of erythrocyte production, because high reticulocyte counts signify increased erythropoiesis and counts of 0 signify a low level of effective erythropoiesis. Reticulocytes can be reported in at least three different ways, which can sometimes be confusing; 1) as a percentage, 2) as an absolute number, or 3) as a corrected value. The immature reticulocyte fraction (IRF) is also gaining popularity among neonatologists [12].

A reticulocyte percentage is the percentage of erythrocytes that stain as reticulocytes. An obvious limitation of this method is that it fails to account for differences in the absolute number of erythrocytes. For example, each of two neonates may have a reticulocyte count of 5%. The neonatologist may be tempted to conclude that the two patients have similar levels of erythrocyte production. However, the first patient is anemic, with an erythrocyte count of $2 \times 10^6/\mu L$, and the other is not anemic, with an erythrocyte count of $4 \times 10^6/\mu L$. The anemic infant actually has only one-half the number of reticulocytes in the circulation (5% of 2×10^6, or 100,000 reticulocytes/μL of blood), as does the normal infant (5% of 4×10^6, or 200,000 reticulocytes/μL). Failure to recognize this pitfall can give the neonatologist the false impression that the anemic patient is mounting an appropriate increase in erythrocyte production. Reporting the reticulocyte count as an absolute number appears to be gaining popularity in neonatal intensive care units. In reporting the results of clinical trials of recombinant erythropoietin administration to preterm neonates this method has been more common for reporting a percentage because it gives a clearer comparison of effective erythropoiesis between groups, despite differences in hematocrit.

A third method of reporting reticulocytes is by using one or two corrections. One type of correction is for hematocrit. The reticulocyte percentage is adjusted to a standard hematocrit, usually 45% (0.45 L/L) and the correction is applied as follows:

$$\text{Corrected reticulocyte count} = \frac{\text{Patient's reticulocyte count (\%)} \times \text{Patient's hematocrit (L/L)}}{0.45\ \text{(L/L)}}$$

This correction is usually appropriate for neonatal patients and provides a better framework for assessing the level of effective erythropoiesis, because it normalizes all values to a standard hematocrit.

Another type of correction is sometimes made for shift reticulocytes. This correction accounts for the observation that whereas reticulocytes generally survive in the circulation for only about 1 day, during increased erythropoiesis even younger reticulocytes can be released from the marrow to the blood (i.e., shifted).

These shift reticulocytes, prematurely released from the marrow into the blood, survive longer than 1 day in the blood before losing their organelles, giving the false impression that the reticulocyte count is high. When reticulocytes survive in the circulation for 2 days, rather than 1 day, the correction for shift reticulocytes is made by reducing the percentage reported in half. To apply this correction, the degree of shift is assumed to be related to the intensity of stimulation of the marrow by erythropoietin. The examiner assumes that the maturation time of the reticulocyte in the circulation is 1 day when the hematocrit is normal, 1.5 days when the hematocrit is reduced moderately, and 2.0 days when tile hematocrit is reduced markedly. With a hematocrit of 20%, the physician assumes a marked erythropoietic effort, causing a shift in premature reticulocytes that survive 2 days in the circulation:

$$\text{Corrected reticulocyte count} = \frac{\text{Patient's reticulocyte count (\%)}}{2.0}$$

The shift reticulocyte count correction is rarely used by neonatologists. The correction for shift reticulocytes is only appropriate when the anemia is the result of hemorrhage or hemolysis, because it assumes that erythrocyte production has increased in response to the anemia. If the anemia is the result of hypoproduction of erythrocytes the reticulocyte count correction for shift reticulocytes would not be valid. Because relative hypoproduction of erythrocytes is thought to be a common component of anemia in preterm infants, the use of this correction might be misleading.

The immature reticulocyte fraction (IRF) is a measure of the proportion of reticulocytes with intense reticulum staining. A threshold is set, and the fraction of reticulocytes staining above that threshold reflects the "young" reticulocytes. Thus a high IRF is used as a parameter of brisk erythropoiesis. Thus, after erythropoietin treatment, the IRF might increase; otherwise an erythrocyte transfusion the IRF might fall.

On the day of birth, normal term infants have reticulocyte values of 4–7% and absolute reticulocyte counts of 200,000–400,000/μL [13]. Infants delivered prematurely have somewhat higher reticulocyte counts; values of 6–10% and absolute counts of 400,000–550,000/μL are common [14]. In healthy neonates, reticulocyte levels fall markedly over the first few days of life. By the fourth day the reticulocytes can be 0–1%, with an absolute count of 0–50,000/μL.

98.2.6 Erythrocyte Morphology

Using erythrocytes suspended in wet preparations, Zipursky enumerated the various morphologic forms of erythrocytes in term and preterm infants and in adults [15]. He observed considerable heterogeneity among erythrocytes of infants, particularly preterm infants. Whereas 78% of erythrocytes from adults were discocytes, only about 40% of the erythrocytes of neonates had this characteristic shape. Morphologic abnormalities of erythrocytes of neonates have also been reported using other methods. For instance, interference-contrast microscopy shows pits or craters on the surface of about 2.6% of erythrocytes of healthy adults. In contrast, about 25% of the erythrocytes of term infants and about one half of the erythrocytes of preterm infants have pits [16]. It is believed that these pits are cytoplasmic vacuoles and represent hypofunction of the spleen or reticuloendothelial system, but their significance is not clear.

98.2.7 Site of Sampling

The hematocrit, hemoglobin concentration, and erythrocyte concentration of newborn infants vary, somewhat predictably, according to the vascular source from which the sample is obtained (Table 98.2) [17–22]. In general, erythrocyte values are higher (i.e., more concentrated) when samples are obtained by lancing capillary beds than when drawn from an artery or a vein or obtained from an indwelling arterial or venous catheter. The specific anatomic site of the vein, artery, or capillary bed sampled appears not to affect the results. For instance, values obtained from a femoral vein are equivalent to those obtained from a scalp vein [21] and values from an umbilical artery are equivalent to those from a radial artery. Similarly, values from a capillary bed, such as from a heel, toe, or finger, are also equal if perfusion of those sites is similar [21].

As a general rule, capillary blood obtained from a neonate's poorly perfused extremity is a poor source on which to base clinical decisions. The erythrocyte values from such a source are about 15% (range of 5 to 25%) above those of simultaneously obtained venous or arterial blood. Capillary values obtained from a poorly perfused extremity can erroneously indicate the diagnosis of polycythemia and can erroneously exclude anemia. The amount that a capillary hematocrit is elevated above a venous or arterial hematocrit cannot be accurately predicted in individual cases, but in general, appears to vary with perfusion of the extremity.

When a CBC is obtained from a sick neonate with poor skin perfusion, the neonatologist interpreting the value should realize that a capillary hematocrit is less informative and less reproducible than a venous or an arterial hematocrit. Oh and Lind [20] and Lindercamp and colleagues [22] found that infants of the shortest gestation generally have the largest difference in capillary and venous hematocrits (Table 98.2).

Table 98.2 The effect of site of blood sampling on hematocrit or blood hemoglobin concentrations of neonates

Author	Year	Anatomic sites of comparison	Average difference
Valquist	1941	Capillary *vs* femoral vein	Capillary 10% higher
Oettinger & Mills	1949	Great toe *vs* internal jugular	Capillary 21% higher
Mollision	1951	Capillary *vs* venous	Capillary 5% higher
Oh & Lind	1966	Heel *vs* scalp or femoral vein	Capillary 15% higher*
Moe	1967	Patients with erythroblastosis fetalis; heel *vs* umbilical vessel	Capillary 25% higher
Linderkamp	1977	Capillary *vs* umbilical vessel	Capillary 10-21% higher
Rivera & Rudolph	1982	Heel *vs* antecubital vein	Capillary 12-20% higher
Thurlbeck & McIntosh**	1987	Heel *vs* UAC	Capillary 15% higher

UAC umbilical artery catheter.
* The capillary hematocrit was only 5% higher when the heel was warmed ** Preterm infants, 24 to 32 weeks' gestation, with respiratory distress.

Studies by Oh and Lind [20] and by Moe [21] indicate that warming the extremity before lancing the capillary bed can result in a better correlation between capillary and venous hematocrits. However, when a neonatologist feels it important to detect changes in serial hematocrit samples, it should likewise be important to keep a uniform site of sampling (i.e., all venous or all arterial). When comparing serial hematocrits of an ill neonate, it is useful to have the sample site documented.

98.2.8 Blood Sampling Relative to Delivery

About 100 mL of blood usually can be withdrawn from the placental vessels at term by using the technique of catheterizing the vessels and washing out the blood [23]. Direct venipuncture of the placental end of the umbilical vein after cord clamping generally yields somewhat less blood (50–60 mL). The blood within the umbilical cord and placenta constitute about one third of the entire circulating blood volume of the fetus.

Normally, after a term fetus is delivered, the umbilical arteries constrict in response to the increasing PO_2, retarding the flow of blood from the neonate into the placenta. The umbilical vein, however, fails to significantly constrict, permitting blood to flow in a direction partly controlled by gravity – from the placenta to the neonate or vice versa. In the time between delivery and clamping of the umbilical cord, the direction of the umbilical venous blood flow usually is from the placenta to the infant. However, if the neonate is held in a position significantly above the placenta, blood can flow through the umbilical vein from the neonate to the placenta [24, 25].

98.3 Leukocytes

The 1972 edition of *Holt's Pediatrics* stated, "the blood count is of relatively little help in the diagnosis of sepsis neonatorum" [26]. This was a prevalent teaching of the time, and when the first cases of group B streptococcal infection were reported, no mention was made of blood leukocyte counts or differential cells counts in these neonates. The notion that the CBC was not a useful test to perform on neonatal patients was based on the extreme variability of neutrophil counts in neonates, and the apparent lack of correlation between the blood neutrophil findings and the presence of infection.

Attitudes about the utility of CBCs of neonates began to change after publications of Xanthou [27], Zipursky et al [28], Akenzua et al [29], and Manroe et al [30]. These reports showed differences in blood neutrophil concentrations in groups of infected compared with noninfected neonates, and they pointed out that neutropenia, accompanied by a high ratio of immature neutrophils (bands and metamyelocytes) to total neutrophils (segmented neutrophils and band neutrophils and metamyelocytes) was particularly common in neonates with sepsis.

98.3.1 Site of Sampling

Neutrophil concentrations are lower in blood drawn from an umbilical artery catheter (or other arterial line) than in blood simultaneously drawn by venipuncture or capillary stick [31]. Arterial values are about 75% of the venous or capillary values. After moderate exercise in the form of chest physical therapy, the leukocyte counts increase to about 115% of baseline, but this is not accompanied by a change in the differential count. Lower neutrophil concentrations in arterial blood are observed in experimental animals [32]. The explanation appears to be related to the differences in blood flow of arterial compared with capillary or venous blood. The pulsatile flow of arterial blood appears to "push" the larger cells toward the periphery of the vessel, whereas the leukocytes and erythrocytes are more uniformly mixed in capillary and venous circulation.

98.3.2 Leukocyte Counts in Term Infants

In 1979, Manroe and associates from the University of Texas Southwestern Medical Center in Dallas published reference ranges for blood neutrophil concentrations in neonates and for the proportion of circulating neutrophils that were immature (nonsegmented) (Fig. 98.9). They reported values for

neonates of 26–44 weeks' gestation and with birth weights of 660–5000 g [30]. The data were obtained from 1974–1976, when the survival of very-low-birth-weight (VLBW) infants was 42% (compared with 79% a decade later). The number of VLBW infants represented in the Manroe study was limited. This publication constituted a landmark; for the first time, neonatologists could determine with some confidence whether their patient had a blood neutrophil count that was low, normal, or high [33].

Neonates at high altitude, such as those in the NICUs of Colorado [34], Utah [35], and New Mexico [36], have a higher range of neutrophil counts than do those reported from near sea level (Dallas) [30]. Fig. 98.10 shows the reference range of blood neutrophils among neonates at altitude and Fig. 98.11 shows the altitude and sea level ranges superimposed to directly compare the differences.

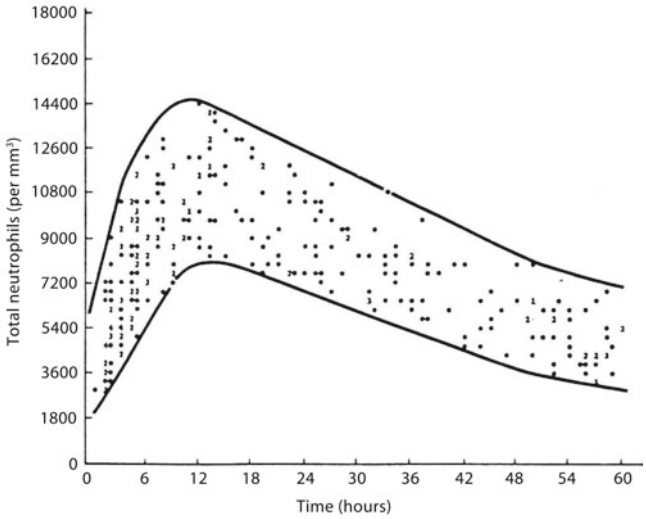

Fig. 98.9 Manroe chart. Reference range for blood neutrophil concentration of normal newborn infants in Dallas Texas during the first 60 hours after birth. Dots represent single values and numbers represent the number of values at that same point. Reproduced from [30], with permission

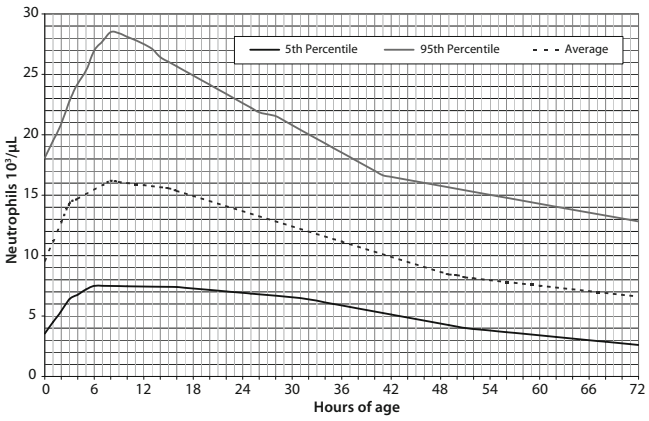

Fig. 98.10 Reference range for blood neutrophil concentrations during the first 72 hours after birth of term and near-term (> 36 week gestation) neonates. A total of 12,149 values were used in this analysis. The 5th percentile, mean, and 95th percentile values are shown. Reproduced from [35]

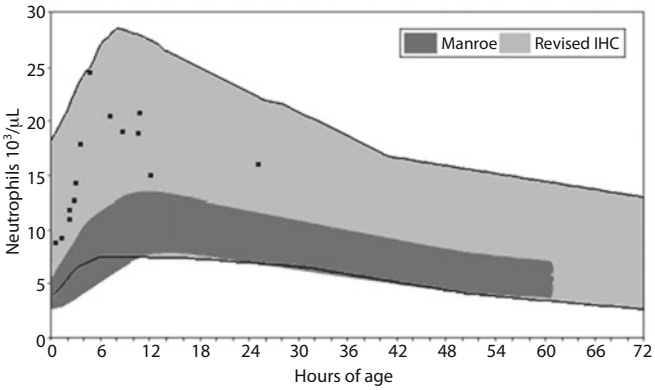

Fig. 98.11 Reference range for blood neutrophil concentrations, superimposed Manroe and Schmutz curves (revised IHC, Intermountain Healthcare)

98.3.3 Leukocyte Counts in Preterm Infants

Several studies suggested that the normal values reported by Manroe might not be applicable to VLBW infants. One of the first of these was a report by Coulombel and coworkers of 132 neonates with CBCs obtained during their first 12 hours of life [37]. Blood was sometimes obtained from capillary samples and other times from umbilical venous or arterial catheters. All 132 infants had a clinical reason for obtaining a CBC, although this report excluded those with culture-positive evidence of bacterial infection. The researchers observed an inverse relationship between gestational age and blood neutrophil concentration.

In 1982, Lloyd and Oto reported serial leukocyte counts from 24 preterm infants, all less than 33 weeks' gestation [38]. They observed that the group's blood neutrophil concentrations were lower than those reported by Manroe (predominantly term infants). In 1994, Mouzinho and colleagues reported the results of 1799 blood leukocyte and differential counts obtained from 193 VLBW infants [33]. After excluding counts from neonates with perinatal and/or neonatal complications, values from normal VLBW neonates were displayed and compared with the Manroe reference ranges (Fig. 98.12). Infants of younger gestations had reference ranges for blood neutrophils that differed significantly from those of larger, older neonates. The VLBW infants had the same upper limit boundary for absolute neutrophil count of the larger, older infants, but their lower limit boundary was significantly less than the boundary of the older infants. Reference ranges for neonates 28–36 weeks gestation are shown in Fig. 98.13 and for neonates < 28 weeks gestation in Fig. 98.14.

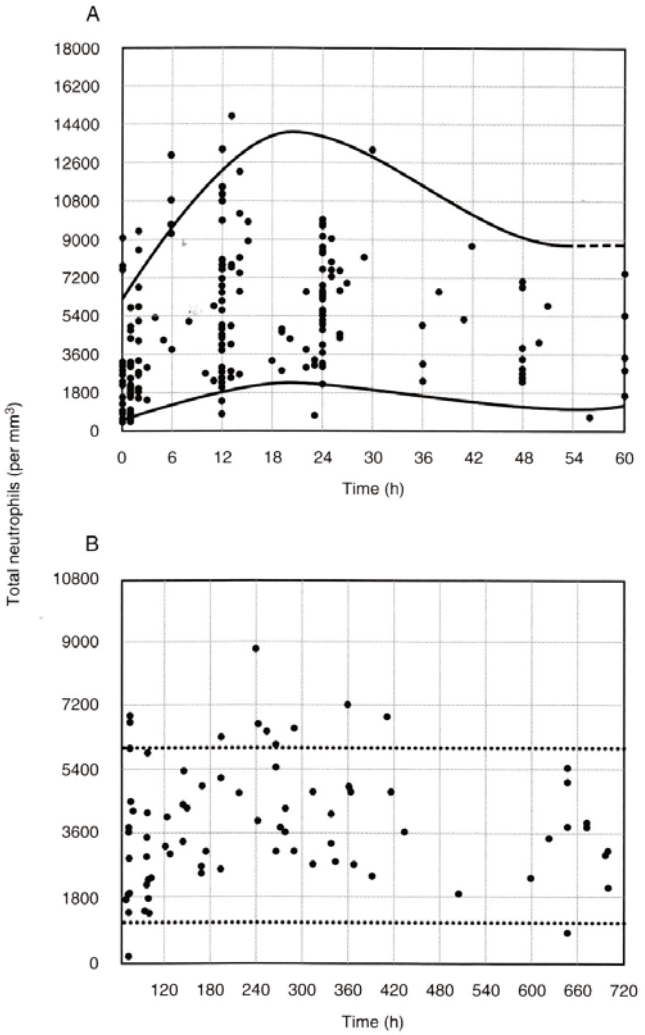

Fig. 98.12 Mouzinho chart. Reference range for absolute blood neutrophil concentration of very low birth weight infants. Dots represent single values. Reproduced from [33], with permission

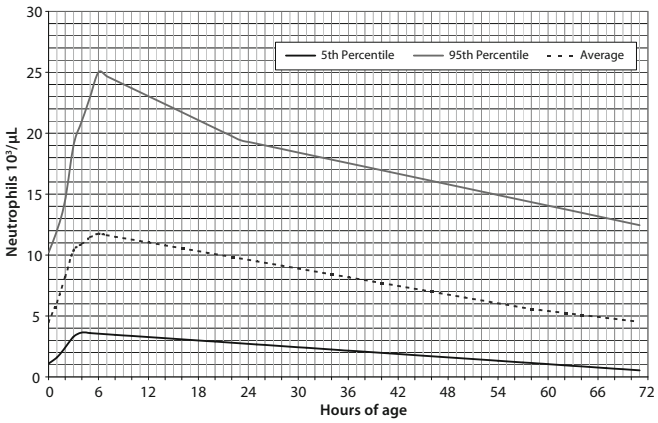

Fig. 98.13 Reference range for blood neutrophil count during the first 72 hours after birth of neonates 28 36 weeks' gestation. A total of 8,896 values were used in this analysis. The 5th percentile, mean, and 95th percentile values are shown. Reproduced from [35]

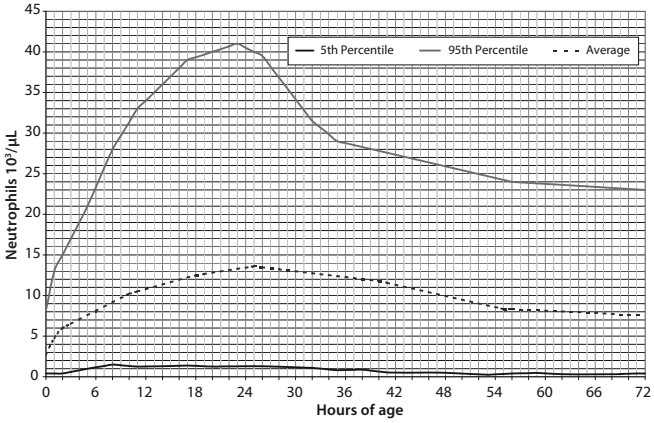

Fig. 98.14 Reference range for blood neutrophil count during the first 72 hours after birth of neonates < 28 weeks gestation. A total of 8,896 values were used in this analysis. The 5th percentile, mean, and 95th percentile values are shown. Reproduced from [35]

98.3.4 Eosinophils

Eosinophilia is not a rare finding in the neonatal intensive care unit. In growing preterm infants a nonspecific low-grade eosinophilia is so common as to warrant the label "eosinophilia of prematurity" [39, 40]. Despite its relatively frequent occurrence, the causes and significance of this condition are uncertain. The concentration of eosinophils in the blood can increase to very high concentrations without a concomitant increase in the concentration of other leukocytes. This attests to a unique controlling mechanism. The mechanisms regulating eosinophil concentrations in blood are reviewed in Chapter 105 and the material given here relate only to reference ranges for eosinophils.

Defining the normal range of eosinophils in the blood of neonates has been attempted in relatively few studies. Medoff and Barber [41] reported that during the first 12 hours of life, absolute blood eosinophil concentrations range from 20 to 850/µL. On the basis of this report, eosinophilia on the first day of life would be defined as an absolute blood eosinophil count of more than 850/µL. Xanthou reported that, by the fifth day of life, eosinophil concentrations had a much greater range than on the first day, with values of 100–2500/µL [42]. By day 5, eosinophilia would be diagnosed if the count was more than 2500/µL. Our group recently complied reference ranges for neonates using electronic CBC records of over 80,000 neonates in a multihospital healthcare system [43]. As shown in Fig. 98.15, the blood concentration of eosinophils gradually increases over the interval of 22 to 42 weeks' gestation. As shown in Figure 98.16, the reference range over the first month following birth increases slightly, with the 95% value at birth being about 1200/µL, and at one month being 1500/µL.

According to the reports of Medoff and Xanthou, absolute eosinophil counts of less than 20/µL on the first day of life and

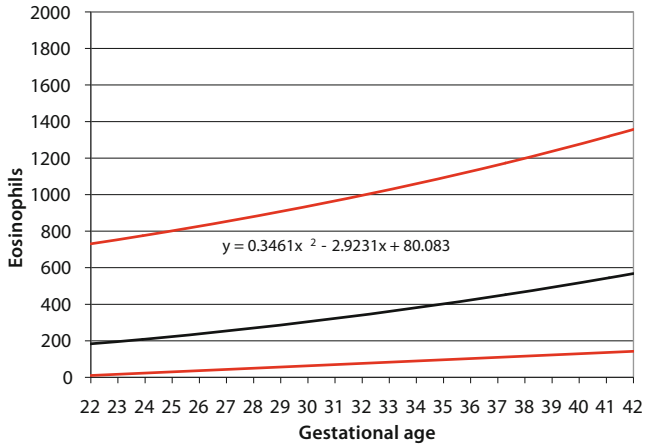

Fig. 98.15 Reference range for blood concentration of eosinophils on the day of birth from 22–42 weeks' gestation. The 5th percentile, mean, and 95th percentile values are shown. Reproduced from [43]

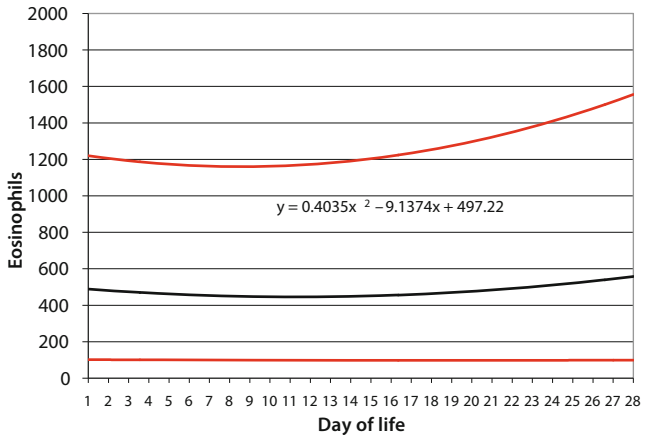

Fig. 98.16 Reference range for blood concentration of eosinophils during the first 28 days following birth. The 5th percentile, mean, and 95th percentile values are shown. Reproduced from [43]

less than 100/μL after the first 5 days should define eosinopenia. However, the diagnosis of eosinopenia in the neonatal intensive care unit is made rarely, because so many apparently normal infants have no eosinophils identified on individual differential cell counts. In contrast to this statement, Burell suggested that a complete lack of eosinophils in neonates should be considered an abnormality [44], our findings [43] are consistent with that conclusion. He found that a complete absence of eosinophils was common in infants who fared poorly and subsequently died. He reported that on the day of death there was generally a complete absence of blood eosinophils. Bass reported that blood eosinophil concentrations declined rapidly in the presence of bacterial infection [44].

By defining eosinophilia as blood concentration of more than 700/μL (a definition of eosinophilia sometimes used in adult medicine), Gibson and coworkers [45] found that about 75% of preterm infants develop eosinophilia. This may be because 700/μL is a value well within the reference range for neonates. They reported that eosinophilia in preterm infants generally occurs during a period in which an anabolic state is established. This anabolic state and the accompanying eosinophilia usually are seen in the second or third week of life, and the eosinophilia persists for many days and sometimes for weeks. Portuguez-Molavasi reported that at least one episode of eosinophilia (defined as an absolute eosinophil count of more than 1000/μL) occurred in 35% of all infants admitted to an intensive care nursery [46]. Craver reported that eosinophils can be seen in the cerebrospinal fluid weeks after intraventricular hemorrhage [47] but the significance of this finding is not clear and this finding did not correlate with blood eosinophil counts.

98.4 Platelets

98.4.1 Site of Sampling

Thurlbeck and McIntosh [48] reported no difference in the platelet counts drawn from umbilical arterial lines, venipunctures, or heel stick. Therefore, unlike the case for neutrophils and erythrocytes, it does not appear to matter what vascular source is used for drawing a platelet count. On that basis, it is common practice to obtain platelet counts of neonates from the most convenient sampling site, without concern of a potential sampling-site difference.

98.4.2 Reference Ranges for Platelet Counts and Mean Platelet Volume on the Day of Birth

In adults, the reference range for platelet count is > 150,000/μL and < 450,000/μL [49]. This same range has been used to define thrombocytopenia and thrombocytosis in neonates. However, the data suggest a more complex situation. The reference range for platelet count at birth, between 22 and 42 weeks' gestation, is shown in Fig. 98.17 [50]. Platelet counts as low as 100,000/μL can be normal for neonates > 29 weeks' gestation and counts above 35,000/μL are high for neonates > 29 weeks. While the blood concentration of platelets increases gradually between 22 and 42 weeks, the mean platelet volume (MPV) does not. The MPV averages 8 fL, with a range of about 7 to 9.5 fL.

Following birth platelet count gradually increases. As shown in the upper panel of Fig. 98.18, the reference range over the first 90 days after birth has the appearance of a sign-wave. The first wave could be the result of thrombopoietin (and perhaps other megakaryopoietic stimulators) surging after birth. The explanation for the second and third wave of peak platelet counts is not clear. Accompanying the increase

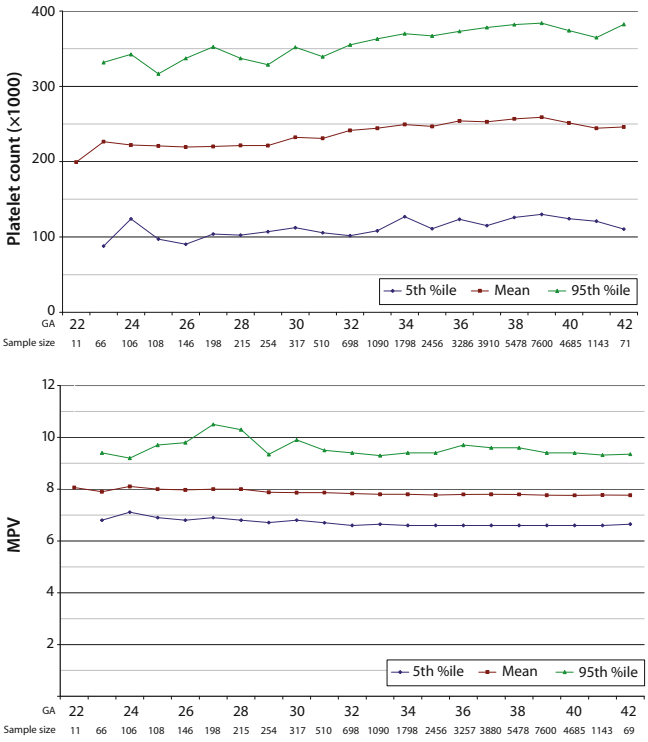

Fig. 98.17 Reference range for platelet count (*upper panel*) and mean platelet volume (*lower panel*) on the day of birth, for neonates delivered at 2242 weeks gestation. Values are shown for the 5th percentile, mean, and 95th percentile. Reproduced from [51]

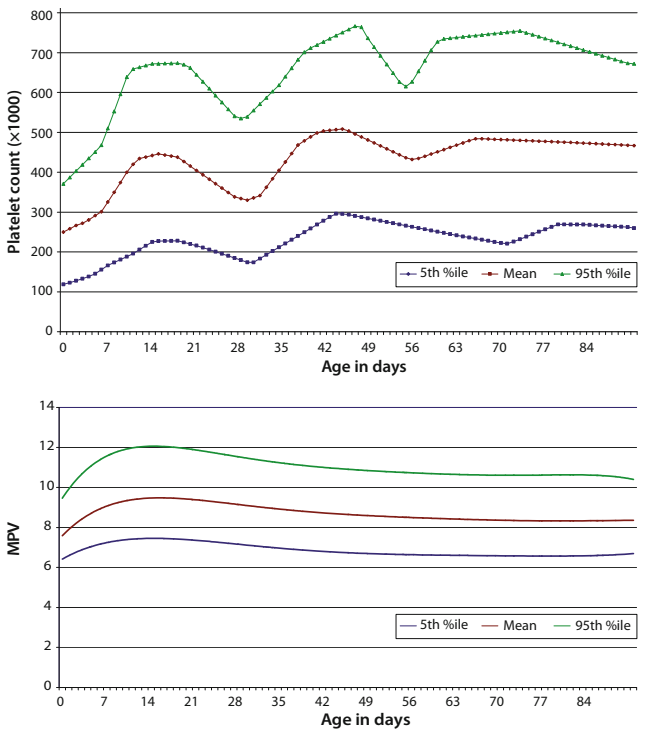

Fig. 98.18 Reference range for platelet count (*upper panel*) and mean platelet volume (*lower panel*) during the first 90 days following birth. Values are shown for the 5th percentile, mean, and 95th percentile. Reproduced from [51]

in platelet count after birth is an increase in MPV (shown in the lower panel of Fig. 98.17). At two weeks after birth, the reference range for platelet count is about 7.5–12 fL, but platelet size gradually falls to that at birth at a month or two.

98.4.3 Reticulated Platelets, Platelet Distribution Width, Platelet 'Crit and Immature Platelet Fraction

Additional information on platelet characteristics can sometimes be useful in managing a thrombocytopenic neonate. Relatively new tests include the reticulated platelets count, which requires flow cytometric capability, and the platelet distribution width and platelet 'crit, which can be performed on routine hematology analyzers (Beckman-Coulter), and the immature platelet fraction, which can be performed on other hematology analyzers (Sysmex Inc.).

Newly synthesized platelets generally have a higher content of ribonucleic acid than platelets that have been circulating for several days. These newly produced platelets can be recognized by flow cytometric analysis after RNA staining with thiazole orange. Such platelets have been called reticulated platelets to reflect their similarity to reticulocytes [52]. Between 3 and 5% of the circulating platelets of healthy term neonates and LBW infants are recognized as reticulated platelets. Peterec and colleagues [52] reported that infants of less than 30 weeks gestation had reticulated platelet percentages about twice those of term infants, although with a very large variation (8.8% ± 5.1%). Joseph and associates [53] observed that adults (n = 18) had a slightly but significantly higher percentage of reticulated platelets than did healthy term neonates (n = 42). They observed no difference in reticulated platelet count between infants depending on mode of delivery.

The platelet distribution width, like the erythrocyte distribution width, is a measure of the distribution of various sized platelets in the circulation [54].

98.5 Bone Marrow

A bone marrow aspirate or biopsy occasionally is useful in providing information not otherwise available. In cases of a persistent cytopenia, a neonatologist might want information relating to the state of production. For example, a persistent thrombocytopenia could be the result of decreased platelet production or of increased platelet destruction or use. A marrow examination can sometimes help differentiate these conditions because in cases of decreased platelet production, the marrow contains few megakaryocytes, but in cases of increased platelet use, the proportion of megakaryocytes should be increased.

Table 98.3 Differential cell counts of nucleated cells aspirated from the bone marrow of normal infants at term and from healthy adult men

Cell Types	Term Neonates (% [mean ± SD])	Health Adults (% [mean, 95% CI])
Nucleated erythroid		
Proerythroblast	0.1 ± 0.1	0.6; 0.1–1.1
Basophilic erythroblast	0.2 ± 0.2	1.4; 0.4–2.4
Polychromatic erythroblast	13.1 ± 6.8	21.6; 13.1–30.1
Orthochromic erythroblast	0.7 ± 0.7	2.0; 0.3–3.7
Neutrophil		
Promyelocytes	0.8 ± 0.9	3.3; 1.9–4.7
Myelocytes	3.9 ± 2.9	12.7; 8.5–16.9
Metamyelocytes	19.4 ±4.8	15.9; 7.1–24.7
Band neutrophils	28.9 ± 7.6	12.4; 9.4–15.4
Segmented neutrophils	7.4 ± 4.6	7.4; 3.8–11.0
Myeloid: erythroid ratio	4.3 : 1	3.1:1; 1.1–5.2:1
Eosinophils	2.7 ± 1.3	3.1; 1.1–5.2
Basophils	0.1 ± 0.2	0.1
Monocytes	0.9 ± 0.9	0.3; 0.0–0.6
Lymphocytes	14.4 ± 5.5	16.2; 8.6–23.8
Megakaryocytes	0.1 ± 0.1	0.1
Plasma cells	0.0 ± 0.1	1.3; 0.0–3.5
Undifferentiated blasts	0.3 ±0.3	0.9:0.1–1.7
Unknown of damaged cells	6.0 ± 3.2	

Data for term neonates: from Rosse C et al (1977) Bone marrow cell populations of normal infants: the predominance of lymphocytes. J Lab Clin Med 89:1225–1240; and from Shapiro LM, Bassen FA (1941) Sternal marrow changes during first week of life. Am J Med Sci 202:341–354.

Unfortunately, bone marrow aspirates of neonates are often diluted with peripheral blood, which can lead to misinterpretations about the state of production. This limitation can be minimized by taking care to withdraw the smallest possible marrow sample, thereby not overdiluting the specimen with blood. The limitation can be further minimized by using a biopsy technique [55].

Marrow is accessible from essentially any bone in the neonate. We have found that the flat part of the proximal tibia is a simple and reliable site from which to obtain a marrow sample, because this site is easy to stabilize, the landmarks are obvious, and the amount of tissue between the skin and the bone is small. The (right-handed) operator holds the tibia firmly in a gloved left hand, while the right hand is used to prepare and sterilely drape the area and then to inject 1% lidocaine as a local anesthetic. Lidocaine (typically 0.1 mL without epinephrine) is injected subcutaneously, holding the needle in place for several seconds, after the needle is advanced to the bone and another 0.1 mL is injected into the periosteum. A few minutes later, the operator holds the 19-gauge, 1/2-inch needle (Osgood marrow needle fitted with a trocar) in the right hand and twists it carefully through the anesthetized periosteum with the mid-tibia clearly marked directly between the left thumb and forefinger. The needle is directed toward the middle of the tibial shaft to enter the middle of the marrow cavity. The needle is correctly positioned when it is solidly in place and when, on aspiration, marrow enters the aspirating syringe [55].

If only a microscopic analysis of the bone marrow cells is to be accomplished, only a few drops of marrow should be aspirated. If flow cytometry, a karyotype analysis, or a hematopoietic progenitor cell culture is needed, more cells are needed, and up to 0.25 mL of marrow can sometimes be aspirated. Volumes exceeding this amount, particularly in very small preterm infants, essentially always draw significant amounts of peripheral blood through the marrow and result in a dilute specimen, which may be difficult to interpret.

Little information is available on bone marrow biopsies in neonates. Although the issue of cellularity is much more accurately assessed with a biopsy specimen than with an aspirate, neonatologists have been reluctant to perform this somewhat more invasive procedure, particularly on very small and ill neonates. We have found success in obtaining marrow needle biopsies, even in the smallest preterm infants in our neonatal intensive care unit, by using a modification of the aspiration method [55].

After the operator feels the Osgood needle penetrating the cortex of the tibia, the stylet (trocar) is withdrawn. The hollow needle is then advanced into the marrow space an additional 3–5 mm. Advancing the needle without a stylet cores a small segment of marrow into the needle. A syringe is then attached to the needle hub, and minimal suction is applied for about 1 second. This process helps secure the smallcore marrow biopsy specimen within the needle. The syringe and the attached needle are then withdrawn, and the core of marrow is pushed out of the needle by reintroducing the stylet. The core can be placed into fixative and subsequently sectioned and stained, or it can be smeared on coverglasses.

Anticipated results of bone marrow differential counts are shown in Table 98.3. Studies in 1941 and 1952 used marrow aspirates obtained from normal neonates, but this practice is no longer permitted. The normal values for marrow nucleated cell concentrations in neonates are available, but normal values for laboratory tests that were devised since 1952 (e.g., concentrations of hematopoietic progenitor cells) are not available [55]. In modern practice, bone marrow aspirates are only performed on neonates if a clinical reason exists to do so or the patient is enrolled in a study that requires a bone marrow study. These subjects therefore are not strictly normal subjects, and the results should not be considered normal values.

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99

Pathophysiology of Coagulation and Deficiencies of Coagulation Factors in the Newborn

Rodney P.A. Rivers

99.1 Introduction

The main points of difference between neonatal and adult hemostasis are as follows:

- Platelets show significant differences in function
- Bleeding times are shorter through increased activity of von Willebrand factor (VWF)
- Increased tissue factor (TF) availability from monocytes and microparticles
- Many procoagulant and inhibitor levels are gestationally dependent
- Thrombin generation equivalent to adult
- Reduced procoagulant and inhibitor reserve
- Relatively impaired fibrinolysis
- In both, normal screening test results do not equate with normal hemostasis.

The principal function of the components making up the hemostatic system is to limit hemorrhage at sites of vascular injury. Subendothelial fibroblasts demonstrate strong TF protein staining and TF mRNA hybridization, as do pericytes surrounding capillaries. These sources of TF act as a "hemastatic envelope", triggering coagulation when exposed to blood.

Activated endothelial receptor expression and VWF secretion are essential for normal platelet participation in hemostasis. VWF is secreted as ultralarge multimers (ULVWFM). They are readily cleaved to shorter, less reactive multimers by the enzyme ADAMTS-13. In plasma, VWF has a half-life of approximately 12 hours and binds to FVIII, protecting it from activated protein C (APC).

Platelet adhesion, aggregation, granule release, surface phospholipid expression and contraction make critical contributions to hemostasis.

The plasma-based clotting factors form membrane-bound complexes of proteases and cofactors which achieve rapid amplification of the coagulation process. The phases of thrombin generation (TG) have been labelled as the initiation, amplification or propagation and termination phases (Figs. 99.1, 99.2, 99.3). Clot-bound thrombin activation of

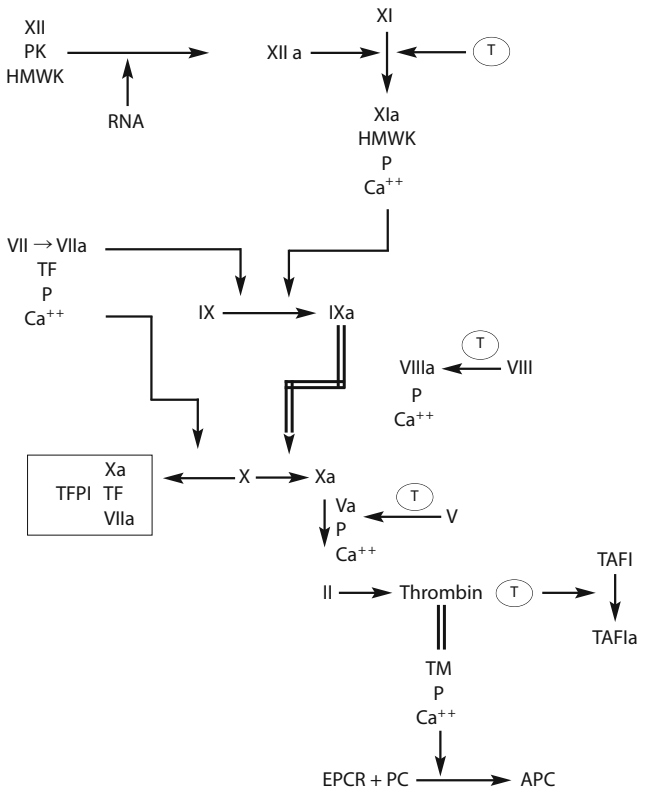

Fig. 99.1 Pathways of coagulation activation illustrating the importance of the intrinsic pathway of FX activation once the TF pathway of initial activation is shut down by TFPI complex formation. *PK* plasma kallikrein, *HMWK* high molecular weight kininogen, *P* phospholipid, *TF* tissue factor, *TFPI* tissue factor pathway inhibitor, *TAFI* thrombin-activatable fibrinolysis inhibitor, *TAFIa* activated thrombin-activatable fibrinolysis inhibitor, *TM* thrombomodulin, *EPCR* endothelial protein C receptor, *PC* protein C, *APC* activated protein C

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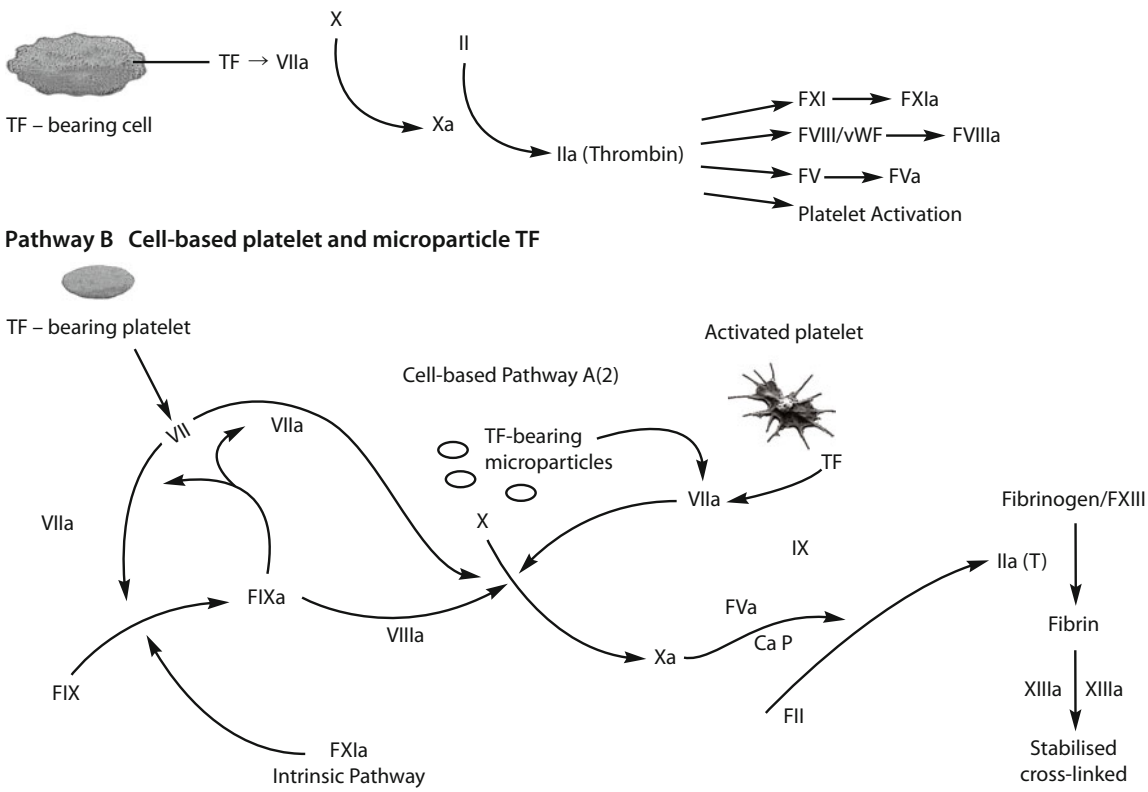

Fig. 99.2 Pathways of coagulation activation. Initiation membrane-linked activation pathway A and subsequent amplification intrinsic pathway B with involvement of platelet and microparticle tissue factor (*TF*) and membranes

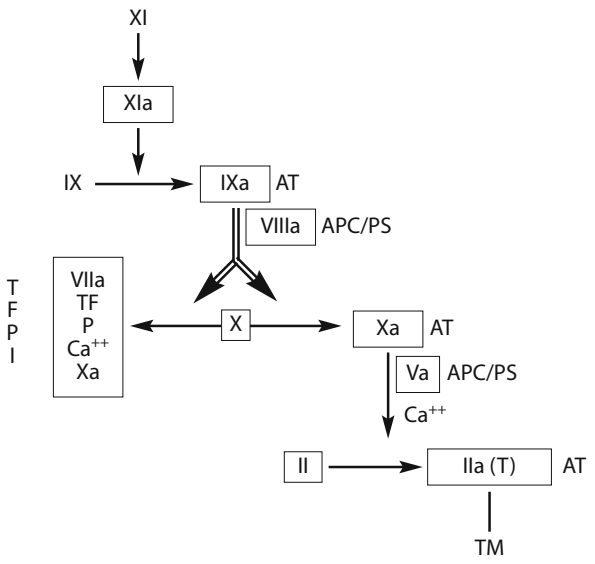

Fig. 99.3 Regulation of coagulation pathways: effectors of procoagulant inhibition. Sites of inhibition of thrombin generation and termination of thrombin activity on binding to thromboglobulin. *AT* antithrombin, *APC* activated protein C, *PS* protein S, *TFPI* tissue factor pathway inhibitor, *TM* thrombomodulin

FVIII results in continued FXa and thrombin formation, important for stable clot formation. This re-supply via FVIII is missing in hemophilia A.

The physiologic inhibitor of the TF-VIIa complex is TF pathway inhibitor (TFPI). Once this pathway of FX activation is blocked, the FIXa –VIIIa complex or intrinsic pathway becomes the principal pathway of FX activation. In the termination phase, free thrombin loses its procoagulant and platelet activating activity on binding to thrombomodulin (TM) and to the abundant antithrombin (AT).

99.2 Vitamin K-Dependent Proteins

During carboxylation of their precursor molecules in hepatocytes, vitamin K (VK) is oxidized to epoxide and is recycled via an oxidation-reduction pathway for reuse in its reduced, hydroquinone form, in the γ-carboxylation reaction (Fig. 99.4).

In hemostasis, two classes of VK-dependent proteins are identifiable, one of procoagulants: Factors II, VII, IX and X,

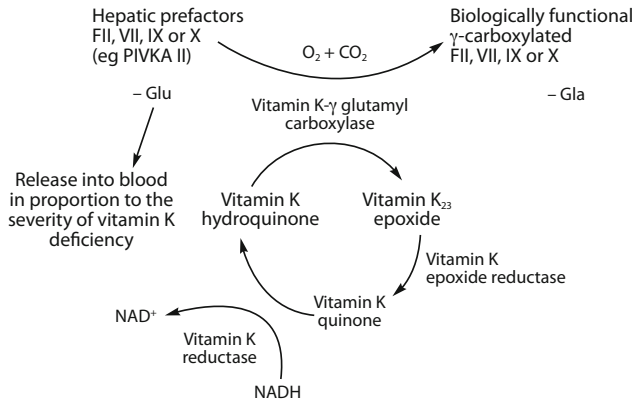

Fig. 99.4 Vitamin K - Metabolism and dependent factors. *PIVKA* protein induced by vitamin K absence or inhibition

and one of inhibitors: protein C (PC), protein S (PS) and protein Z (PZ). The serine protease domain of Factors II, VII, IX, X and of PC becomes functional after cleavage of specific peptide bonds. Neither PS nor PZ are serine proteases. All depend on γ-carboxylation of glutamic acid residues for their biologic activity. Reduced carboxylation in the absence of sufficient VK is associated with reduced activity. Precursor molecules in liver microsomes serve as immediately available substrates for the γ-carboxylase enzyme once VK becomes available. Precursors are released into the circulation as proteins induced by vitamin K absence (PIVKAs). With a 70 hour half life for PIVKA-II, its measurement can be used to diagnose previous VK deficiency even after VK has been administered.

99.3 Inhibitors in Plasma

Once the initiation phase is shut down by TFPI, the generation and activities of thrombin become regulated by AT and by α_2-macroglobulin (α_2-M) inhibition of the procoagulant serine proteases and by PC. The α_2-M is an anticoagulant when AT levels are low but it inhibits PS/APC complex formation and so can act as a procoagulant. Once bound to TM, thrombin activates PC on an adjacent endothelial protein C receptor (EPCR); PC is then released as APC. The EPCR is also a receptor for FVII/VIIa, facilitating FVIIa endothelial endocytosis. Soluble EPCR is found in plasma; it reduces PC availability for the membrane EPCR and inhibits APC inactivation of FVa thereby acting as a procoagulant (Fig. 99.5). PC has a half-time of some 6–8 hours in plasma, APC a half-time of only 15–30 minutes. APC proteolytic inactivation of FVa downregulates thrombin generation; inactivation of factor VIIIa diminishes the amplification phase. Rates of inactivation are enhanced by protein S. PS also acts as a cofactor in TFPI inhibition of FXa (Fig. 99.6). The heparin cofactor II (HCII) proteinase only inhibits thrombin; activity is enhanced by dermatan sulphate. Protein Z forms a complex with the protein Z-dependent protease inhibitor (ZPI) enhancing ZPI inhibition of FXa on phospholipid surfaces.

Fibrinolysis of cross-linked fibrin is initiated by tissue plasminogen activator (tPA) released from endothelial cells which, like urokinase plasminogen activator (uPA), activates plasminogen to plasmin, a process that is tightly controlled. On the clot surface, tPA and plasmin are relatively inaccessible to plasminogen activator inhibitor-1 (PAI-1)

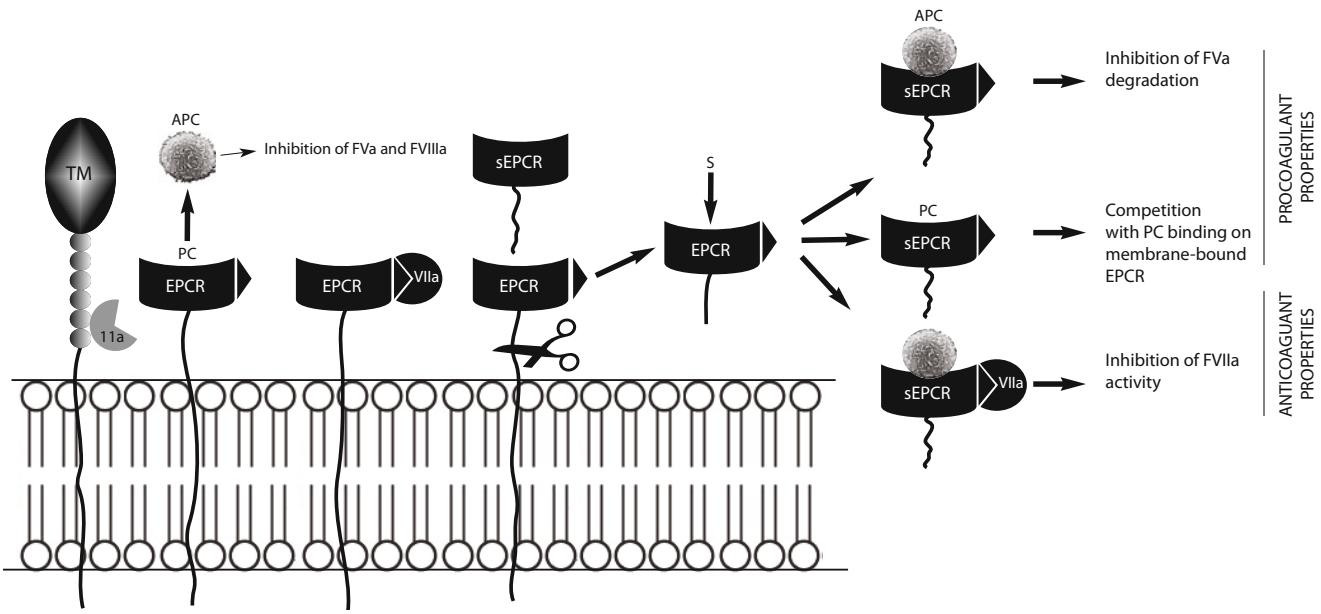

Fig. 99.5 EPCR and TM in coagulation/endothelium. Roles of thromboglobulin, of endothelially bound protein C receptor and of released plasma EPCR in downregulation of thrombin generation. *TM* thrombomodulin, *APC* activated protein C, *EPCR* endothelial protein C receptor, s*EPCR* soluble endothelial protein C receptor, *PC* protein C

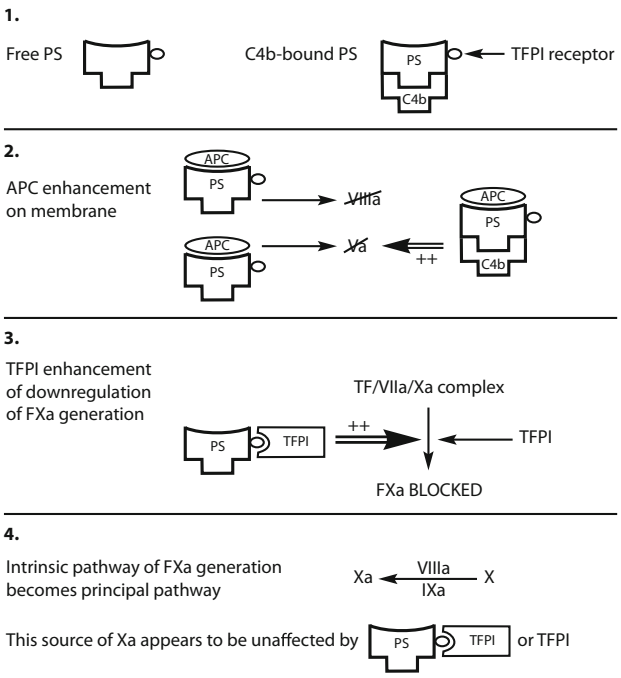

Fig. 99.6 Mechanisms of protein S enhancement of thrombin generation inhibition. Protease digestion of FVa and FVIIIa by protein C is enhanced by protein S. PS is active whether free or bound to C4b-binding. Enhancement of TFPI inhibition of the TF/VIIa/Xa complex is independent of protein C, but FXa generated by the intrinsic pathway is not inhibited. *PS* protein S, *TFPI* tissue factor pathway inhibitor, *APC* activated protein C

and α_2-antiplasmin (α_2-AP). In plasma, both are rapidly neutralised. Excessive activation, or a failure of inhibition, results in fibrinogen degradation (Fig. 99.7).

99.4 Hemostasis in the Neonate

Bleeding disorders may be life-threatening and rapid evaluation is essential. A detailed family history is required and consideration of clinical circumstances is needed. Is the baby apparently well or is the bleeding possibly a secondary phenomenon?

99.4.1 Investigations

Not all components of hemostasis are evaluated by routine screening tests and so "normal" test results for gestation and postnatal age do not equate with the presence of normal hemostasis. In 1991, the International Society for Thrombosis and Haemostasis Scientific and Standardisation Subcommittee on Neonatal Haemostasis published its recommendations for establishing normal ranges for neonates [1]. Data from cord blood studies may not equate with results obtained neonatally where difficult sampling may lead to TF contamination and slow withdrawal from catheters may lead to activation with soluble fibrin (SF) formation *in vitro*.

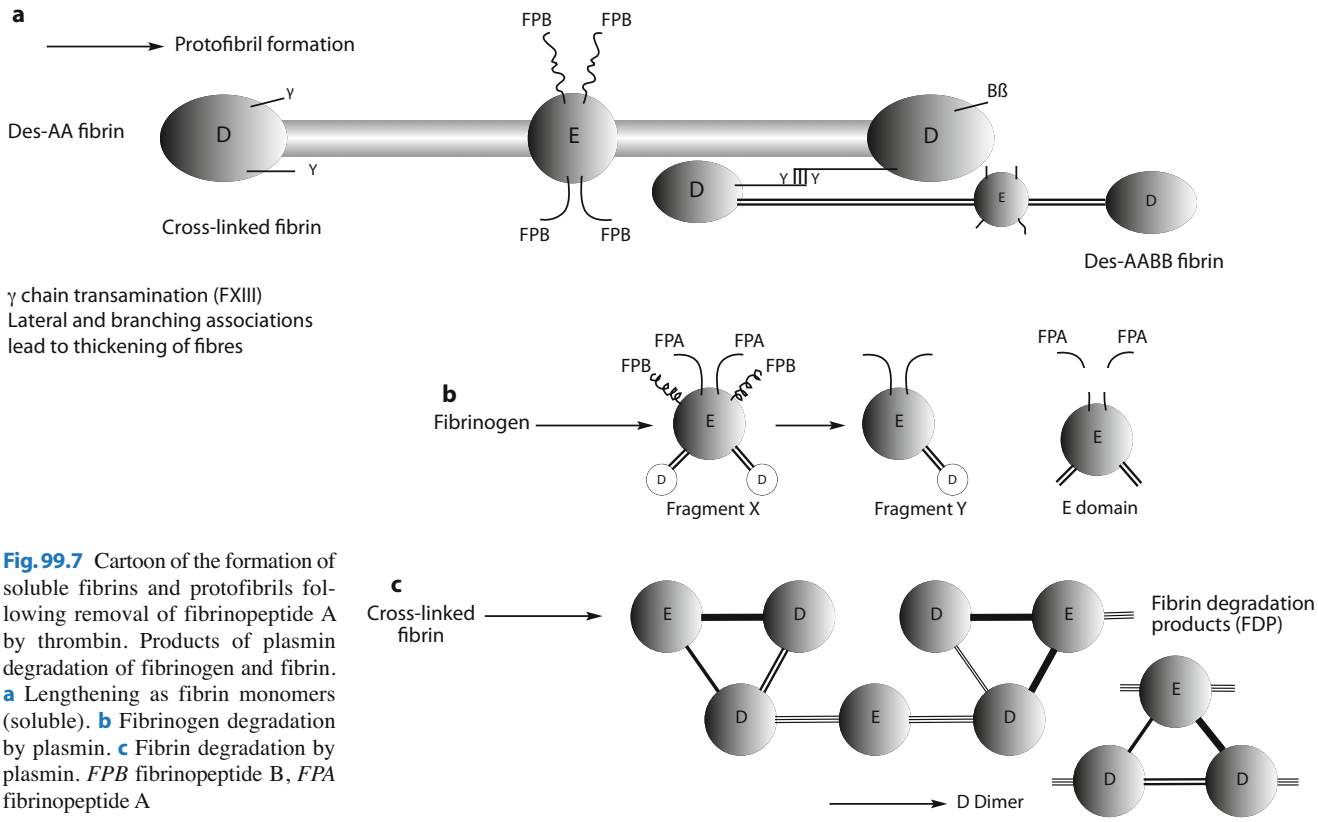

Fig. 99.7 Cartoon of the formation of soluble fibrins and protofibrils following removal of fibrinopeptide A by thrombin. Products of plasmin degradation of fibrinogen and fibrin. **a** Lengthening as fibrin monomers (soluble). **b** Fibrinogen degradation by plasmin. **c** Fibrin degradation by plasmin. *FPB* fibrinopeptide B, *FPA* fibrinopeptide A

Many problems may affect the validity of screening tests. These include incomplete anticoagulant mixing with clotting in the sample, hemolysis and lipemia which interfere with optical end point determinations, and contamination by heparin or the presence of an inhibitor as suggested by prolongation of an activated partial thromboplastin time (APTT) test on a 1:1 mixture of control and test plasmas. Heparinase degrades unfractionated or low-molecular weight heparin and, if added to a final concentration of 25 mg/mL, neutralises up to 2 U/mL, allowing accurate performance of the screening tests and of specific factor assays. Excessive dilution by anticoagulant (the normal 1 in 10 dilution assumes a hematocrit of 45%) may result in exaggeratedly prolonged times as neither the prothrombin time (PT) nor the APTT are linearly related to factor activities. The Clinical and Laboratory Standards Institute (CLSI) recommends adjustment of the ratio anticoagulant to sample volume according to the formula: volume of anticoagulant = (100 – PCV)/(595 – PCV) where PCV (packed cell volume) is expressed as a percentage. This should apply to hematocrits over 55% [1]. The high level of proteolytic activity after birth can result in *in vitro* formation of fibrinogen degradation products (FDP) and degradation of FV and FVIII by the plasmin present; proteases from activated granulocytes degrade FXIII, FIX, FX, FVII and FII. Incorporating ε-aminocaproic acid (AB Kabi, Stockholm) in the anticoagulant inhibits these effects. Sample cooling below 18°C induces FVII activation and loss of VWF.

Spontaneous aggregation of platelets due to leakage of adenosine diphosphate (ADP) from erythrocytes is described and a relationship to the hematocrit documented. Reported low platelet counts should always be checked microscopically.

The evolution of hemostasis development has been discernable from studies on fetal [2] and newborn plasmas at differing gestational and postnatal ages [3–5]. Problems in the diagnosis of congenital factor deficiencies arise from the normally low levels of several of the proteins involved. Most are measurable by 10 weeks' gestation; some display functional changes relating to differences in molecular composition.

FV, FVIII, fibrinogen and platelet count are present at close to adult values by 24 weeks' gestation whereas other components including high molecular weight kininogen (HMWK), the VK-dependent factors II, VII, IX and X, FXII and plasminogen approximate to 20% of adult levels by 28 weeks' gestation rising to around 50% at term.

Factor XIII shows no clear gestational dependence, remaining at 50–75% of adult from 30 weeks' gestation to term, but reaching adult values by 3 weeks' postnatal age.

At birth, FVIII levels are skewed towards the higher levels and fibrinogen levels continue to rise afterwards. The prolonged thrombin time (TT) obtained even when measures to prevent *in vitro* fibrinogenolysis are taken, results from the "fetal" form of fibrinogen. Removal of the increased sialic acid content of this fetal form leads to a correction of the prolonged TT as does adding calcium ions to the thrombin in the test.

Levels of VWF are increased at birth; the VWF is present in larger sized multimers than normal and displays increased activity in promoting platelet adhesion. The raised level persists for the first 3 months of life. Limited studies on ADAMTS-13 in the newborn have given discrepant results with both adult equivalent and reduced levels being reported.

99.4.2 Inhibitors of Coagulation

Protein C is present in a fetal form but function does not appear to be affected; it is present at around 50% of the adult value, increasing slowly after birth but not achieving adult values until the early teens.

Alpha$_2$-M, which is elevated above adult levels in neonates, has been shown to inhibit APC in a dose-dependent fashion therefore acting as a procoagulant.

Protein S exists in its free form in the newborn since the C4b-binding protein is virtually absent but the level is reduced. Its interaction with protein C may be affected by the level of α_2-M [6]. The free TFPI level is also reduced to some 50–65% of adult values. The PZ level has been reported as being low in neonates, particularly in preterms or in those born to mothers with pre-eclampsia; deficiencies in adults have not been reproducibly associated with pathological outcomes.

Antithrombin is at only some 30% of adult at under 30 weeks of gestation rising to 50% at term. α_1-antitrypsin (α_1-AT) and α_2-AP are somewhat reduced in proportion to gestation, but may exceed adult levels at term. A relative inhibition of fibrinolysis predominates throughout gestation.

99.4.3 Postnatal Changes

In the postnatal period, factor levels rise at differing rates; in term neonates, Factors VII and XIII attain adult levels by 3 weeks' postnatal age. Factors II, IX, XI, prekallikrein, AT and PC levels rise more slowly and in both term and preterm infants born at 30–36 weeks' gestation, adult levels are not attained until 3–6 months of age. In a separate study, the levels of Factors V and VII in infants born at gestations of 24–27 weeks increased to higher levels at 6 months postnatal age than those in infants born at greater gestational ages.

TM plasma concentrations are increased in the newborn with preterm neonates having higher levels than those born at term, the highest levels being found in those ventilated for severe respiratory distress possibly reflecting pulmonary endothelial damage.

Thrombin inhibition has been thought to be principally effected by the adult level of α_2-M since AT levels are low. With the demonstrated increased binding of thrombin to HCII, the overall inhibitory capacity in plasma appeared similar to the adult although thrombin inhibition was slower. Some inhibition may also be provided by a placental anticoagulant in cord

blood with similarities to dermatan sulphate. It catalyses thrombin inhibition by HCII. AT survival times, measured following exchange transfusion, are shorter than in adults possibly because exchange transfusions activate coagulation [7].

In studies of TG and on the time course of prothrombin fragment 1 and 2 formation, α_2-M inhibition was not evident. Changes made in α_2-M concentrations failed to result in changes in free TG or in prothrombin activation in neonatal plasmas. It was suggested that thrombin inhibition by α_2-M may be too slow to prevent feedback activation by the generated thrombin.

Clot strength has been assessed using thrombelastometry to provide information on maximum clot firmness (MCF) and on fibrin polymerization. MCF was significantly lower in cord versus adult whole blood reflecting "fetal" fibrinogen's impaired polymerization.

99.4.4 Fibrinolysis

In the neonate, all major fibrinolytic proteins demonstrate age-dependent concentration variations [8]. Marked changes in the components occur following birth. TPA and PAI-1 are below adult levels in cord blood whereas in both term and preterm neonates, tPA, uPA and PAI-1 are transiently increased above the adult value on day 1.

Plasminogen levels are some 20% of adult at 28 weeks and 50% at term, rising to the normal adult range by 6 months with α_2-AP levels being at 80% of adult at term. Fetal plasminogen exists in two major glycoforms with increased mannose and sialic acid content displaying slower activation kinetics than adult forms. Functionally they bind less well to cellular receptors and the plasmin formed from it has less enzymatic activity. Mean α_2-AP levels are around 80% of adult at term. The inhibition of the two fetal plasmin isoforms by α_2-AP (plasmin-antiplasmin [PAP] complexes) is slower than that of the adult plasmin variants. The other principal plasmin inhibitors, are α_2-M and α_1-antitrypsin. Although elevated levels of PAP complexes in healthy newborns derive from increased production of plasminogen activators at birth, inhibition of fibrinolysis predominates in the plasma of the newborn beyond day one.

The changes associated with advancing gestation were initially reported in the 1970s and have become known as developmental hemostasis, a term coined by Andrew [9]. Published reference ranges are available from data derived in the 1980s and early 1990s by Andrew, colleagues [8] and by others [10]. The preterm infants studied were all equal to or more than 30 weeks of gestation.

99.4.5 Clot Translucency and Strength

Over the years very different techniques have been utilised to record the end point of clot formation and published reference data may not be applicable to reported results from a neonate under investigation now. Differing reagents and machines used by laboratories reduce the utility of past published reference ranges. Comparisons with more recently acquired data have revealed important differences [11] (Table 99.1). When screening throws up an abnormal result, it is essential to have access to appropriate reference ranges; not only may further investigation be indicated but planned procedures may be delayed causing anxiety to parents and staff. Variation in the prothrombin time has been minimized by reporting the PT as an international normalized ratio. No such standardization exists for the activators and phospholipids in the APTT.

Table 99.1 Analyser comparisons. Comparison between prothrombin time results obtained using A, an ACL analyser and Thromorel, Behring [9] with B, those obtained using an STA Compact analyser with Neoplastine Cl reagent [11], and between a FVIII assay A using an ACL one stage factor assay and an in-house deficient plasma [9] with results B using an STA Compact analyser one stage assay with STA deficient plasma [11]

	Day 1 neonates	
	A	B
PT (sec)	15.16 (14.4-16.4)	13.10 (11.6-14.4)
INR	1.26	1.0
APTT (sec)	38.7 (34.3-44.8)	42.9 (31.3-54.5)
FVIII%	182	100

PT prothrombin time, *INR* international normalized ratio, *APTT* activated partial thromboplastin time.

Because of their relative rarity and because of the disorders so frequently encountered by them, true normative data for preterm neonates below 30 weeks' gestation do not exist.

It is hoped that reference ranges will be produced from large centers so that other centers using similar apparatus and reagents may be able to consult them when faced with abnormal results. Factor assays and screening tests need to be interpreted using gestational age and postnatal age-adjusted ranges.

99.4.6 Neonatal Platelets

Reference ranges for platelet counts in term and preterm neonates show that counts differ from the accepted adult normal range of 150,000/μL to 450,000/μL. Below 30 weeks' gestation, the 5th centile is 104,200/μL and in the late preterm and term population, it is 123,100/μL. The mean platelet volume is quite similar to that in adults (7–9 fl) with a mean of 8 fl (range 7–9.5 fl) [12]. Increased numbers of circulating megakaryocyte progenitors and of mature megakaryocytes are found. Circulating precursors are proportional both to the numbers in marrow and to the blood platelet count. Newly synthesised platelets have an increased RNA content and can be identified by flow cytometry. In non-thrombocytopenic neonates, the proportion is similar to that in adults.

Thrombopoietin is detectable in the plasma of newborns of all gestations. Levels are similar to baseline adult levels in term infants (140 pg/mL), with a wide range. In the preterm, levels have been reported to be higher.

99.4.7 Functional Aspects

It can be difficult to avoid activation of platelets in cord blood samples; pre-activation during birth has not been found to be the cause of the hyporeactivity observed. Aspects of neonatal platelet function have been repeatedly reviewed [13, 14]. Platelet receptors for adhesive proteins, GP1a/II (collagen), GP1b/IX/V (VWF) and GPIIb/IIIa (fibrinogen, fibronectin, VWF) are present on fetal and cord blood platelets with receptor concentrations being similar to those on adult-derived platelets. The number of α_2-adrenergic receptors is approximately half that on adult platelets and this is not due to receptor occupancy by catecholamines in cord blood. By 2 months of age, levels are similar to those on adult platelets. The diminished response to thromboxane analogues is not a consequence of a reduction in receptors but is due to attenuated downstream signalling.

99.4.8 Bleeding Time

Templates constructed to create small skin incisions have been utilised to study neonates. Results obtained indicate that bleeding times are similar to or shorter than those in adults. Both the existence of the more active ULVWFM in the newborn and the higher hematocrit contribute.

99.4.9 The Platelet Function Analyser 100 (Dade Behring)

Citrated whole blood is aspirated through an aperture and the time to occlusion of the aperture (150 μm) in the membrane coated with fibrillar type 1 collagen in the presence of either 10 μg epinephrine bitartrate or 50 μg ADP is measured. This is referred to as the closure time. Closure times are shorter for newborn than adult blood.

99.4.10 Aggregometry

Generally, aggregation to a variety of agonists is decreased. The differences are magnified in platelets from preterm neonates. The relevance of such findings to *in vivo* hemostasis has been thrown into question by a report claiming to use a more physiological set of stimuli in a whole blood experiment. Using a combination of collagen and endogenous thrombin as stimulants, maximal aggregation and lag time to the start of aggregation were similar in neonatal and adult samples [15]. This result was attributed to the faster generation of thrombin in neonatal blood and possibly offers an explanation for the "abnormally" short bleeding time of neonates. An increased response to ristocetin reflects the increased level and enhanced function of neonatal VWF. Studied using the cone/plate analyzer, a correlation between demonstrated platelet function and gestation has been reported.

99.4.11 Flow Cytometry

Requiring only small volumes with minimal manipulation, platelet responsiveness can now be followed postnatally. Platelets from both term and <30 weeks' gestational age preterm infants have reduced levels of activation markers following stimulation. In normal infants, adult responsiveness is seen by day 10. P-selectin expression following stimulation is further reduced in preterm newborns in proportion to their gestation. Agonist-induced secretion of granule components is reduced in both term and preterm platelets due to reduced function of receptor-mediated transduction pathways (Fig. 99.8).

99.4.12 Procoagulant Activity

The percentage of platelet-derived microparticles (MPs) is higher in preterm than in term newborn blood. MP generation and membrane flipping leading to phosphatidyl serine (PhS)

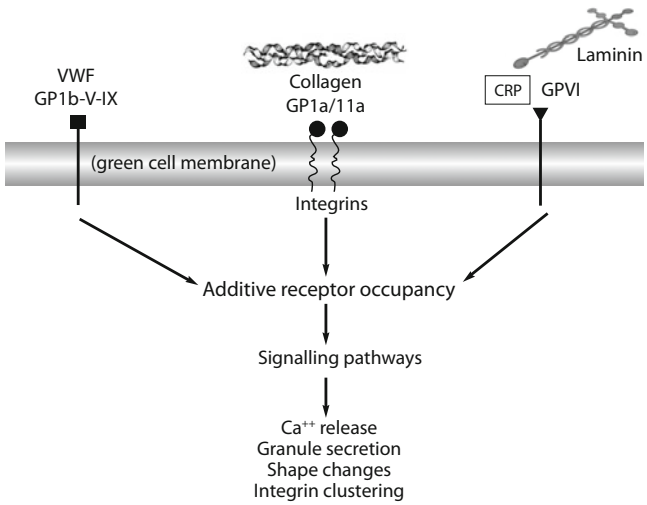

Fig. 99.8 Platelet adhesion receptors and signalling. A summation of platelet receptor occupancy is required for full platelet activation and granule secretion. *CRP* collagen-related peptide, *GP* glycoprotein

expression are both increased in term and in preterm platelets compared to adult platelets. Functionally, thrombin generation is supported [16].

99.4.13 Effects of Drug Exposure

A wide range of drugs including anti-inflammatory agents, antimicrobials, anti-depressants, β blockers and alcohol may cross the placenta and affect fetal and neonatal platelet function and may cause bleeding; in a few instances, drugs including indomethacin and nitric oxide used in neonatal management affect platelet function.

99.4.14 Evaluation of Hemostasis

Conventional tests utilising the strong activators as in the PT and APTT tests only reveal deficiencies in the procoagulant pathways; they provide no information on how the balance between procoagulant and inhibitor concentrations might affect TG in the neonate.

In the early studies of TG using strong activation, TG in neonatal plasma had been found to be some 30–50% of the peak value generated in adult, the poor response being attributed to the low levels of FII encountered in the newborn.

By manipulating the levels of inhibitors including TFPI, AT and PC and by activating the PC with soluble thrombomodulin, the effects on TG using low concentrations of lipidated TF for activation have been studied. TG in cord-derived plasma was found to start earlier due to faster FX activation and to be comparable with that generated in adult plasma, the free thrombin generated being approximately 90% of the adult value. APC was found to have a greater effect on prolonging the clotting time than did increases to AT [6, 17]. Along with evidence from thrombelastography, where clotting is often accelerated, one is led to conclude that the newborn may not be as hemostatically compromised as originally thought. That may be why well neonates exposed to surgery or trauma appear hemostaticly competent. The high levels of tissue factor availability in the newborn may also be contributing to this observed hemostatic competency [18]. However, the neonatal hemostatic components have little in the way of reserves when challenged by excessive activation; bleeding and thrombosis may ensue. In sick neonates, the shortened half-lives and reduced production of procoagulants may rapidly result in the development of a hemorrhagic diathesis.

Presentations of unsuspected hemostatic failure may be revealed following trauma, surgery or may appear to be spontaneous. Major bleeds or continued oozing from the cord or from puncture sites may be the first signs of hemostatic compromise.

Pseudohemorrhage as swallowed maternal blood from the stomach or stools can be distinguished from fetal blood using the Apt test in which a pink colour in 1% sodium hydoxide is retained by fetal but not by adult hemoglobin.

99.4.15 Coagulation Investigation in the Neonatal Period

Acquisition of a detailed family history is imperative. When there is a positive family history, the potential risk should the neonate have acquired the defect along with knowledge as to whether the diagnosis can be made in the neonate have to be taken into account when planning investigations and in determining whether follow-up investigations beyond six months of age may be required. This may be especially needed for the detection of milder congenital defects. Babies born to mothers on warfarin, anti-convulsants or anti-tuberculous drugs should

Table 99.2 Results of laboratory investigations into neonatal coagulation disorders

Condition	PT	APTT	TCT	Fibrinogen	Platelets	Diagnostic/confirmatory investigation
Inherited disorders						
Hemophilia A	N	↑	N	N	N	FVIII assay
Hemophilia B	N	↑	N	N	N	FIX assay
vWD (type 3)	N	↑	N	N	N/↓	FVIII/vWF assay
FVII deficiency	↑	N	N	N	N	FVII assay
FX deficiency	↑	↑	N	N	N	FX assay
FXIII deficiency	N	N	N	N	N	FXIII assay
FV deficiency	↑	↑	N	N	N	FV assay
FXI deficiency	N	↑	N	N	N	FXI assay
Fibrinogen def.	N/↑	N/↑	↑	↓	N	Fibrinogen assay
Platelet disorder	N	N	N	N	N/↓	Morphology functional evaluation
Acquired disorders						
Vitamin K deficiency	↑	↑	N	N	N	PIVKA II assay
Disseminated intravascular coagulation	↑	↑	↑	↓	↓	D-Dimers
Liver disease	↑	↑	N/↑	N/↓	N/↓	FV & FVIII assays

be screened because of inhibitory effects on VK. Awareness of conditions associated with disseminated intravascular coagulation (DIC) is important.

Samples that have been particularly difficult to obtain or where the tubes are over or under filled should be rejected. Screening tests should include a prothrombin time, which can normally be prolonged by up to 3 seconds in term infants but is more prolonged the shorter the gestation. Adult values for the PT should be achieved by day 4 in all but the most preterm neonates. The international normalised ratio (INR) should be calculated. An activated partial thromboplastin and mixture time, a thrombin-calcium time, a fibrinogen level and full blood count and platelet count should also be performed.

In the very preterm infant the APTT is normally prolonged but the mixture time will reveal whether an inhibitor such as heparin or dermatan sulphate is present. Adult APTT values are achieved by 2–6 months postnatally. The thrombin calcium time is slightly prolonged in the newborn. The Reptilase time with calcium is slightly prolonged in the neonate but is abnormal in some dysfibrinogenemias. Evidence of *in vivo* thrombin generation can be gained by finding thrombin-antithrombin complexes or soluble fibrin monomers. A D-dimer assay or fibrin degradation product measurement may also be useful if DIC is suspected. The changes associated with various conditions are shown in Table 99.2.

Deficiencies of ADAMTS-13, FXIII and of α_2-antiplasmin are not detected in the routine investigations. FXIII should be evaluated by measurement of the transglutaminase activity following thrombin/calcium activation [19].

Measurements of individual components of the systems involved may be required but antigen levels do not necessarily equate with biological function. Platelet function can be assessed by the platelet function analyzer closure time or by a template bleeding time, the latter having the disadvantage of being operator dependent. Platelet glycoprotein (GP) expression is fully developed in term and preterm neonates; by using flow cytometry, deficiency states in receptor expression can be identified. Investigation of the parents' platelet function may be helpful.

In the disordered proteolysis of DIC, routine tests if done sequentially would be expected to reveal falls in FV and FVIII whereas in metabolic liver disease or VK deficiency they are conserved, unless DIC has been triggered by blood loss and shock.

99.5 Inherited Coagulation Disorders

Exposure to surgery poses a considerable threat to an infant when a hemorrhagic diathesis has not been previously suspected. The risks entail complicating or life-threatening hemorrhage and transfusion-transmitted infections from infused plasma-derived products. Immune stimulation leading to inhibitor formation is particularly to be feared.

99.5.1 The Hemophilias

A well term neonate with signs of bleeding and an isolated borderline or prolonged APTT may have a form of hemophilia. Along with von Willibrand's disease, the hemophilias account for over 95% of all inherited bleeding disorders. Hemophilia, as it involves the neonate, has been reviewed by Chalmers [20].

There are three types arising from separate factor deficiencies: hemophilia A (FVIII deficiency), B (FIX deficiency) and C (FXI deficiency). Hemophilia A and B are indistinguishable clinically, both giving rise to bleeding of variable severity.

Prenatal carrier testing can be performed utilising polymerase chain reaction techniques; the molecular genetic testing for hemophilia A has been reviewed by Goodeve [21].

The genes for FVIII and FIX are present on the X chromosome and that of FXI is on chromosome 4. A male infant with abnormal bleeding and normal screening tests except for a prolonged APTT should be screened for FVIII and FIX deficiencies. Both have been described in phenotypic girls.

FXI deficiency gives rise to less severe bleeding but should be considered in all well neonates with bleeding and an abnormal APTT; it is an autosomal recessive disorder.

Even in FVIII or FIX severe deficiency, bleeding in the first week of life is uncommon. In one retrospective review, puncture bleeding (16%) and bleeding post circumcision (30%) were the most common; hemorrhagic shock from liver hematomata in two neonates has been reported.

Intracranial hemorrhage (ICH) is rare and elective cesarean section is not recommended for its prevention. The subject has been reviewed by Ljung [22]. Vacuum extraction was found to be particularly associated with extracranial hemorrhages in the Swedish studies with 69% of 16 cranial hemorrhages occurring in relation to this procedure. Some 50% of those born with hemophilia are not suspected antenatally. Extracranial hemorrhage (ECH) in the form of cephalhematomas and subgaleal hemorrhages can involve massive blood loss and can be accompanied by an ICH which may be clinically unsuspected.

Prophylactic treatment of identified cases of hemophilia is not generally advocated. If an ICH is identified, treatment should continue for 2–3 weeks aiming at 100% concentration over the first 7 days and 50% over the latter part of the course.

As cesarean section does not eliminate the risk of cranial bleeding, normal vaginal delivery for known carriers is generally recommended. The management of carriers and of babies has been reviewed by Street et al [23].

In cases of known or suspected carriers where the fetal diagnosis is unknown, a cord blood sample should be obtained for diagnosis and intramuscular administration of vitamin K should be withheld. Oral vitamin K prophylaxis should be commenced and continued if the diagnosis is confirmed and a cranial ultrasound performed before discharge. Circumcision should be avoided.

Because of the association of a low FVIII level in type III von Willebrand disease (VWD), VWF antigen should be measured in all infants with FVIII activity below 10%.

The FIX level may be as low as 15 IU/dL in normal neonates. Severe and moderately severe deficiency states may be discernible but mild cases cannot be differentiated from those who are not affected and require further evaluation when the child is older unless a genetic diagnosis is possible.

99.5.1.1 Why is Bleeding Uncommon?

Studies have demonstrated that the peak height of TG in FVIII-depleted neonatal plasma is almost as high as that in normal neonatal plasma whereas when adult plasma is similarly depleted of FVIII, TG is only 50% of that observed in normal adult plasma and the time to peak height was 50% longer [24]. Raised TF availability along with the lower levels of TFPI and AT, combined with the PS inhibition by α_2-M, may explain why bleeding in neonates with hemophilia is rare.

99.5.1.2 Management of Bleeding

Management of bleeding has been reviewed by Kulkarni and Lusher [25] and by Ljung et al [22]. Therapy utilising a recombinant product is the preferred option. Alternatively, a monoclonally purified, viral inactivated plasma-derived product may be used. Plasma should only be used when concentrates are unavailable.

Limited pharmacokinetic data in the newborn indicate a shortened half- life for FVIII. In adults the half-life of rFVIII is 11–17 hours but in the neonate it may be as short as 6 hours. The volume of distribution in adults is limited to the plasma volume so that each 1 IU/kg infused raises the plasma concentration by 2%. The volume of distribution in the newborn is increased. On this basis, it has been calculated that an initial infusion of 50–60 IU/kg followed by 8–12 hourly infusions of 25–30 IU/kg should raise the FVIII to adult levels.

Proposals for the use of prophylaxis in order to reduce any bleeding consequences of birth trauma are controversial and there is a lack of evidence-based guidelines. Prophylaxis in preterm infants, two with FVIII deficiency and one with FIX deficiency have been reported. The FVIII was given at 50 IU/kg daily increasing to twice daily with septic episodes.

In the neonate receiving rFIX, the half- life was estimated to be 5.8 hours. Because of a reported very large volume of distribution and this rapid elimination, up to 30 IU/kg each hour may be required to maintain 100% FIX activity. Experience with recombinant FIX in the USA has led to a dose recommendation of a 120 IU/kg bolus followed by 60 IU/kg 12 hourly as the maintenance dose.

Antibody (inhibitor) formation in neonates is rare. The genetic and non-genetic risk factors involved have been reviewed [26]. Although the risk of inhibitor development has been a concern, some evidence points to a reduced risk of inhibitor development with early prophylactic treatment.

99.5.2 Von Willebrand Disease

This bleeding disorder affects males and females and is caused by a quantitative or qualitative deficiency of VWF. It is the commonest inherited bleeding disorder with an estimated prevalence of 0.8–1.3%; in most instances it is inherited in an autosomally dominant fashion. Evidence-based guidelines for diagnosis and management have recently been published under the aegis of the National Heart, Lung and Blood Institute Expert Panel (USA) [27].

There are three major types of VWD: type I, in which there is a partial quantitative deficiency, is the most common. In type II, there is a qualitative VWF defect and in type III there is virtually a complete lack of VWF. The bleeding tendency is usually mild and since levels in the neonate are elevated with the high molecular weight multimers (HMWM) forms, bleeding in the neonatal period is unusual. Type III disease may however present with superficial mucosal bleeding; ICH in this form of VWD is described. Where VWD is associated with thrombocytopenia as in the type 2b subgroup or in the more severe form of type III disease, bleeding is more likely to occur.

Diagnosis of type III VWD is made by finding low levels of both FVIII and VWF antigen and of ristocetin cofactor activity. VWF multimers will also be missing. In the presence of thrombocytopenia, type IIb should be excluded. Because of the normally raised levels in the neonate, other forms of VWD can only be diagnosed by molecular techniques.

99.5.2.1 Management

Recombinant VWF is the treatment of choice although plasma-derived concentrates are licenced for use in the USA. VWF is also contained in viricidally treated FVIII concentrate. The recommended loading dose is 30–60 U/kg with maintenance doses of 20–40 U/kg every 12–48 hours. A trough level of > 50 IU/dL should be maintained for 3–5 days according to perceived risks of re-bleeding.

Desmopressin is contraindicated in the neonate because of the danger of inducing hyponatremia. Platelet replacement may be required in bleeding associated with type IIb disease.

99.6 Rare Inherited Bleeding Disorders

These disorders have been the subject of a recent reviews by Peyvandi [28, 29] and guidelines on diagnosis and management have been produced [30].

They are generally inherited as autosomal recessive traits and severe bleeding is encountered in individuals with either homozygous or compound heterozygous mutations.

99.6.1 Disorders of Fibrinogen

Quantitative and qualitative defects including afibrinogenemia and hypofibrinogenemia (autosomal recessive) and the dysfibrinogenemias (autosomal dominant) have been reviewed [31] and may be identified in the neonatal age group. Afibrinogenemia and the more severe forms of hypofibrinogenemia present most often as bleeding from the umbilical cord; however mucosal bleeding is encountered and ICH in afibrinogenemia has been reported. Significant bleeding would not be anticipated with levels above 0.5 g/L.

The dysfibrinogenemias may be detected following investigation of an abnormal coagulation test result or when screening is carried out against a background of a family history. They are associated both with bleeding and thrombotic disorders but are unlikely to present in the neonate.

99.6.1.1 Diagnosis

Fibrinogen levels are within the normal adult range at birth. In afibrinogenemia, the PT, APTT and TCaT are all markedly prolonged and fibrinogen levels in functional and antigenic assays are undetectable. In hypofibrinogenemia, the level of fibrinogen ranges from 0.20–0.80 g/L and determines the degree of prolongation of the above tests. The TCaT and Reptilase time are the tests most likely to reveal a dysfibrinogenemia, the antigenic levels being higher than levels suggested in functional assays.

99.6.1.2 Management

Virally inactivated fibrinogen concentrates are the treatment of choice in bleeding from afibrinogenemia or hypofibrinogenemia. A minimum level of 0.5 g/L is considered necessary for hemostasis although raising the level to 1 g/L is recommended in a bleeding neonate [30]. The half-life of 3–5 days permits infrequent dosing and prophylaxis from an early age given every 7–14 days has been used to maintain a level above 0.5 g/L. In the absence of concentrate, cryoprecipitate or fresh frozen plasma (FFP) can provide fibrinogen but the risks of viral transmission and of volume overload are greater. Use of fibrin glue and antifibrinolytic agents may need to be considered.

Primary prophylaxis is not generally recommended in afibrinogenemia. Following a severe bleed such as an ICH, secondary prophylaxis aimed at keeping a level of above 0.5 g/L is considered reasonable [30].

99.6.2 FII, FV, FVII, FX, FXI, FXIII and Combined FV/FVIII Deficiencies

Each of these deficiencies leads to a life-long bleeding disorder; the congenital disorders of the VK-dependent factors have been the subject of a review [32]. All the above are associated with a neonatal hemorrhagic diathesis. In a large Iranian series ICH was most commonly associated with FVII and FX deficiencies. The deficiencies of Factor II [33], FV [34], FVII [35], FX [36], FXI [37], and FXIII [19] have each been the subjects of recent reviews.

99.6.2.1 Diagnosis

As presented above, the levels of FII, FVII, FX and FXI are reduced compared with adult values both at term and in a gestationally determined manner. Although severe deficiency states are diagnosable in the newborn, those infants with higher levels may need follow-up re-testing to establish an heterozygote diagnosis. Undetectable FII is not compatible with life and has never been reported in a liveborn baby.

In FV deficiency, the severity of the bleeding tendency is variable and has been attributed to the co-existent finding of a reduced level of TFPI. As FV is at normal adult values at birth, both homozygous and heterozygous deficiency states are usually diagnosable in the neonatal period.

FXI severe deficiency is associated with a spectrum of bleeding severity with some homozygous states being almost asymptomatic.

FXII deficiency, although causing a prolonged APTT, does not give rise to clinical bleeding.

It should be noted that a normal APTT for gestation and age does not necessarily rule out an intrinsic factor deficiency since the high FVIII level can normalise the APTT in the presence of other deficiencies.

99.6.2.2 Management

As recommended above, specific factor concentrates (e.g., FVII, FXI) should be used when available; alternatively, prothrombin complex concentrate (e.g., FII and FX) may be considered. FVII deficiency can be rectified using plasma-derived FVII concentrate at 10–30 IU/kg twice weekly or by rFVIIa in doses of 15–25 μg/kg every 3–6 hours. FV replacement requires the use of virally inactivated FFP raising the level to 15 IU/dL with 15–20 mL/kg followed by 5 mL/kg 12 hourly. The level should be maintained at 25% after a severe bleed. FX deficiency is treated with FFP or prothrombin complex concentrates containing FX, although the high levels of FII, FVII and FIX in these concentrates pose a thromboembolic risk; a freeze-dried concentrate of human FIX and FX (FX P Behring, Germany) has become available which may prove useful in managing this disorder.

99.6.2.3 FXIII Deficiency

Although often presenting with umbilical cord bleeding from 1–19 days after birth when levels are below 5 IU/dL, with levels below 1 IU/dL, up to 80% of cases present in this way. The most feared complication is ICH which is common in severe deficiency. Making the diagnosis is therefore particularly important because monthly infusions of FXIII concentrate or of cryoprecipitate can prevent this complication (see below), the half-life of FXIII being around 30 days.

FXIII is not involved in any of the screening coagulation tests and must be analysed specifically utilising a functional assay.

Neonates diagnosed with FXIII levels below 3 IU/dL should be commenced on a prophylactic replacement regimen of viricidally treated, plasma-derived FXIII concentrate. FXIII distribution is limited to the plasma volume. Each 1 IU/kg infused raises the plasma concentration by 2%. The aim is to keep the pre-treatment trough at > 3 IU/dL. This requires 10 IU/kg every 4 weeks initially with subsequent monitoring. FXIII is also present in cryoprecipitate and in FFP.

99.6.2.4 Hereditary Combined VK Deficiency Factor Disease

This is a very rare autosomal recessive condition arising from mutations affecting either the γ-glutamyl carboxylase enzyme or the VK epoxide enzyme complex. In the severely compromised, bleeding may occur in the neonate with umbilical and intracranial hemorrhages reported.

The PT and APTT are variably prolonged depending on the degree of functional deficit. The condition may be difficult to distinguish from VK deficiency since some response may follow VK administration. Molecular studies may be required.

VK should be administered and ongoing prophylaxis with VK may be required. In those unresponsive to VK, both FFP and factor concentrates have been used.

99.7 Fibrinolytic Deficiency States

Inherited deficiencies of PAI-1 and of α_2-AP (autosomal recessive) are very rare; they can give rise to serious bleeding. They can be screened for using the euglobulin clot lysis time.

99.7.1 PAI-1 Deficiency

The uncontrollable fibrinolysis associated with PAI-1 deficiency can give rise to ICH. Infants have a very short euglobulin clot lysis time in the face of normal screening tests. Relative insensitivity of available assays to activity measurements and in antigen detection makes diagnosis of deficiency difficult. Replacement is by using FFP in combination with ε-amino-caproic acid (EACA) as a fibrinolytic inhibitor. Doses of EACA of 50–100 mg/kg every 6 hours orally can maintain clot stability after replacement therapy.

Alpha-1-AT deficiency can only be replaced with FFP and EACA may be of use in controlling excessive lysis.

99.8 Acquired Factor Deficiencies: Liver Dysfunction and Liver Failure

Liver disease can affect the synthesis of a number of coagulation proteins. Levels of procoagulants and anticoagulants can become reduced resulting both in hemorrhagic and prothrombotic tendencies. Comparisons between FV and FVIII levels are helpful in evaluating the liver dysfunction because both should be present at adult levels and FV is the only one dependent on liver synthesis. Abnormal activation can reduce FVIII. Brief responses to platelet and fibrinogen infusions are indicative of consumption. Unless the cause of the liver failure can be reversed, the associated hemorrhagic diathesis is likely to be fatal.

99.8.1 Management

Correction of the PT for liver biopsy or surgery requires rFVIIa. A dose of 90 μg/kg has been found to be effective. Prolongation of clotting factor half times has been advocated using very low doses of heparin (5–10 U/kg an hour).

99.9 Vitamin K Deficiency Bleeding (VKDB)

In the first published series of 50 cases of what he named hemorrhagic disease of the newborn, Townsend identified four key features distinguishing affected babies from cases of hemophilia: early onset, usually on day 2–3 of life, a self-limited time course, a lack of a family history and the importance of adequate feeds in reversing the condition. The name change recommended in 1999 by the ISTH Pediatric/Perinatal Subcommittee to VKDB indicates the specific etiology and widens the inclusion to babies beyond the 4 week neonatal period [38]. Recognition of the particular dangers from the late onset form has resulted in the widespread introduction of various prophylactic regimens. In VK deficiency the glutamate residues of factors II, VII, IX and X are variably, incompletely γ-carboxylated.

Their measurement has been used to detect degrees of VK deficiency. Cord blood levels of PIVKA II vary through gestation, increasing near term and in pregnancies complicated by pre-eclampsia.

Table 99.3 Newborn and adult liver vitamin K_1 and K_2 values. Vitamin K concentrations and total values in livers from newborns and adults. Values in parentheses are median. Data from [39]

	Vit K_1 (ng/g)	Total Vit K_1 (μg)	Total Vit K_1 and K_2 (μg)
Adult	1.1–21.3 (5.4)	1.7–38.3 (7.8)	33–330
Term newborn	0.1–8.8 (0.1)	0.02–0.91 (0.09)	0.02–0.91
Preterm newborn	0.3–6.0 (1.4)	0.02–0.91 (0.09)	0.02–0.91

The differences between the adult and neonatal liver contents per gram of liver are striking [39] (Table 99.3). No VK_2 menaquinones were detectable in the neonatal autopsy material until approximately 14 days postnatal age; in the adult they make up 75–97% of the total liver VK. The newborn seems totally dependent on VK_1 for sufficiency. Fortification of cow's milk formulae was recommended by the American Academy of Pediatrics in 1971 and continues at around 55–60 μg/L. In breast milk, the principal form of VK is phylloquinone (PhQ) with menaquinone-4 being the next highest. The average concentration of PhQ in colostrum is about 2 μg/L and in mature human milk, about 1 μg/L. Even if taking 200 mL/kg a day, it has been estimated that a baby would receive less than 1 μg VK a day. In one report, ceasing to supplement breast fed babies with formula feeds in the first days of life resulted in an incidence VKDB of 1 in 1200 live births.

The effect of the differences in VK intake between breast and formula fed infants on PIVKA II detection is shown in Table 99.4. PIVKA II was only present in the breast fed groups. MK-4 appears to be synthesised in the mother from phylloquinone. The neonatal protective effects of 20 mg VK given daily for 14 days to mothers on anti-convulsants, where early neonatal bleeding had previously been a feature, were clear. Intramuscular VK_1 (0.5–1.0 mg) given at birth raises hepatic concentrations 1000–5000-fold at 24–48 hours and at 6 days was 1.4 μg/g; by 28 days, levels remained some 200-fold above the levels encountered at birth. There is no evidence that neonatal VK_1 provision enhances dependent factor protein synthesis, so in the preterm infant with gestationally determined low levels of the VK-dependent factors, postnatal VK administration will only prevent an emergence of inactive forms. Factor replacement is the only way to achieve improved hemostasis.

Table 99.4 PIVKA II measurements in breast and formula fed infants. Immunoassay on plasma samples from two groups of infants

		Age (months)		
		1	2	3
Breast fed	Number	62	44	32
Vit K: 2.1 μg/mL	PIVKA II + ve	3	4	2
Formula fed	Number	43	48	45
Vit K: 50 μg/mL	PIVKA II + ve	0	0	0

PIVKA protein induced by vit K absence or inhibition. Data from [50].

VKDB, delineated into three types as described by Lane and Hathaway in 1985 and accepted by the Pediatric/Perinatal Subcommittee of the International Society on Thrombosis and Haemostasis in 1999, includes the division into idiopathic and secondary forms [38]. Amongst the secondary causes are the frequently undiagnosed causes of fat malabsoption such as cholestasis, biliary atresia, α_1-antitrypsin deficiency and cystic fibrosis and a failure to be aware of the effects of drugs given to the mother or infant (warfarin, anticonvulsants and antituberculous agents).

Early VKDB is defined as bleeding due to VK deficiency in the first 24 hours of life; it is usually caused by drugs administered to the mother that interfere with VK metabolism but can arise from conditions causing maternal fat malabsorption.

Classical VKDB occurs between days 2 and 7 and is regarded as idiopathic but often has its origins in the failure to establish adequate early breast feeding [40].

Late onset VKDB extends the period of bleeding from day 8 to 6 months [38]. The peak incidence is between 3 and 8 weeks. It is almost always in exclusively breast fed infants. An impaired secretion of bile salts with mild abnormalities in liver function tests leading to VK malabsorption is often implicated. Although warning bleeds can be a feature, many cases present with intracranial bleeds. In one series, severe ICH was noted in 63% of cases. Of those followed up, some 40% had long-term neurological consequences. Factors contributing to the VKDB are the relatively low VK content of breast milk, the lack of gut microflora that synthesise vitamin K_2 and, in preterm infants, inadequately functional VK epoxide cycles (Fig. 99.4). Various chemicals in the breast milk of mothers living in industrialised countries may be acting as enzyme inducers, interfering with quinone metabolism.

Diagnostic criteria of VKDB are: a prothrombin time ≥ four times the control value plus at least one of:

a. a normal or raised platelet count, normal fibrinogen and absent FDP
b. PT returning to normal after vitamin K
c. PIVKA (usually of FII) level above normal control values

A probable case: PT and APTT prolonged for age plus one of the above.

Use of PIVKA measurements may be helpful in differentiating VKDB from cases of non-accidental injury since retinal bleeds, often considered as pathognomonic of NAI, have been described in VKDB.

99.9.1 Treatment of VKDB

A slow intravenous dose of 1–2 mg Konakion will fully correct deficiency in those less than six months of age. The subcutaneous route can be utilised in the absence of venous access. Higher doses may be indicated for the reversal of warfarin antagonists. Increases in factor levels are detectable within 20 minutes of the intravenous dose, values rising to close to normal for age by 2 hours.

Severe bleeding may require factor replacement as well as the VK until the diagnosis can be confirmed. FFP in a dose of 15 mL/kg or prothrombin complex concentrate, where the dose extrapolated from adult data is 50 units/kg body weight, may be used but these take time to arrive and VK administration should not be delayed. rFVIIa can also be effective in life-threatening hemorrhage at 90 μg/kg.

99.9.2 Prevention of VKDB

Surveys on the incidence of both classical and late onset VKDB have been evaluated in a Cochrane review [41] and have been reviewed by Shearer [42]. A UK survey documented that almost all late onset cases were breast fed babies who had received either no VK or just one oral dose. The authors commented that parental refusal of prophylaxis had become more problematic.

99.9.3 Efficacy of Vitamin K Prophylaxis

The single intramuscular injection of 0.5–1.0 mg of phylloquinone offers better protection than the single or most multiple oral dose regimens. A depot effect is not achieved by intravenous or oral delivery. The move towards oral regimens was triggered by publications in 1990 [43] and 1992 [44] linking childhood cancer to receipt of intramuscular VK with a possible doubling of the risk of leukaemia. Subsequently reviews of data from many surveys have concluded that there is no convincing evidence that vitamin K provided by any route influences cancer risk in children, but an influence cannot be totally ruled out [45, 46].

Many oral regimens were found not to prevent all cases of late onset VKDB. However a Danish regimen used when the Konakion Cremophor preparation was available, of a 2 mg dose at birth followed by a 1 mg dose given weekly for 3 months resulted in no cases being reported over a 7.5 year survey period. In a randomized study in the USA with 67 infants in two groups, 2 mg Konakion MM given orally at birth, at 7 days and at 30 days was at least as effective as a single dose of 1mg intramuscularly at birth. Konakion MM given orally is not predictably absorbed in infants with cholestasis. Concerns remain over bioavailability and over compliance with oral dosing regimens. In the USA, a single intramuscular injection of 0.5–1.0 mg PQ remains the recommended prophylaxis. Low dose oral regimens should be continued for 3 months.

In preterm infants, the reduced activity of the VK epoxide reductase is associated with evidence of hepatic overload at previously prescribed parenteral VK dosages. In a recent trial in which differing dosage regimens in babies < 32 weeks' gestational age were compared, 0.2 mg intravenously or 0.5 mg intramuscularly led to VK_1 2,3-epoxide accumulation whereas 0.2 mg intramuscularly did not. The protection lasted almost 3 weeks following which additional supplementation was needed [47]. A prolonged PT in a preterm infant in the first month of life who has received intramuscular VK at birth is unlikely to be due to VK deficiency.

All infants with late-onset bleeding require testing for associated disorders. The importance of VKDB is reflected in the number of continuing reviews of the subject [48, 49].

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Coagulation Disorders: Risk of Thrombosis in the Newborn

Angelo C. Molinari and Paola Saracco

100.1 Introduction

Newborns comprise the largest group of children developing thromboembolism (TE), due to the peculiarities of their developmental hemostatic systems.

The hemostatic system of the healthy neonate, although immature, is generally in balance; on the other hand, in the sick newborn, especially preterm ones, numerous acquired perinatal and iatrogenic conditions might result in a disturbance between coagulation and fibrinolysis, leading to thrombus formation. Nevertheless, the contribution of acquired prothrombotic disorders in the pathogenesis of thromboembolic disease in newborns remains poorly defined.

Little data is currently available regarding the influence of maternal or fetal genes on thrombotic risk in the fetus and neonate.

Due to the unique peculiarities of their developmental coagulation system, newborns are at highest risk of developing TEs among pediatric patients [1–3].

Recently, several studies based on national and international registries [4–7] have evaluated the role of risk factors for thrombosis both in children and newborns, emphasizing the differences between different pediatric and adult ages.

Nevertheless, at present there is no definitive consensus about recommendations on screening for thrombophilia in newborns with TEs.

The present chapter provides an overview of the current knowledge about the role of inherited, acquired perinatal and maternal prothrombotic risk factors as well as clinical and laboratory investigations in neonatal systemic arterial and venous thrombosis.

For neonatal central nervous system (CNS) thrombotic events, see Chapters 139 and 142.

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100.2 Relevance of Laboratory Investigation of Coagulation in Newborns

Given the significant differences between the plasma factor concentrations in different age groups, a detailed knowledge on the development of hemostasis is critical for the neonatologist/intensivist in order to adapt pharmacological approaches and interpret results from laboratory tests in the neonate with TE [8]. Recently, in the first large scale study since Andrew et al [9–11], determining the age associated numerical changes in coagulation proteins, results clearly demonstrated that the absolute values of reference ranges for coagulation assays in neonates and children vary with analyzer and reagent systems. In this study the authors also present for the first time age related ranges for tissue factor (TF) pathway inhibitor (TFPI), D-Dimer and ETP [8].

Thus, coagulation laboratories should develop age-related reference ranges specific to their own testing systems. Without this, accurate diagnosis and management of neonates and children with suspected bleeding or clotting disorders is not possible.

Additional risk factors, as congenital thrombophilia or critical illness, have to be considered separately from the immaturity of neonatal hemostasis

100.3 Inherited Thrombophilia

The term thrombophilia is used to describe an extensive range of inherited defects in the coagulation system, in the fibrinolytic system, in the endothelial cells and in platelets, which are likely to predispose to thrombosis. Over the last decade the number of identified prothrombotic abnormalities has increased dramatically.

Genetic prothrombotic polymorphisms FV G1691A, also reported as R506Q and commonly called FV Leiden (FVL), Factor II G20210A (PRT G20210A) mutation and

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

the homozygous TT genotype of the methylentetrahydrofolate reeducates (MTHFR) C677T polymorphism, are established inherited thrombophilic condtions [12]. The FVL mutation is the most common genetic prothrombotic risk factor, with a prevalence of 4–6%; the presence of the PRT G20210A mutation (1–2% of Caucasians) has the potential to increase circulating concentrations of FII by 15–30%; homozygous mutations of MTHFR C677T (9–19% of Caucasians) produce a defect in the remethylation of homocysteine into methionine, with the end result being hyperhomocysteinemia. Currently, prospective clinical trials employing vitamin therapy are ongoing to determine whether hyperhomocysteinemia is a risk factor for atherothrombosis. Thus, the association between the MTHFR C677T gene mutation and the risk of pediatric thrombosis remains controversial [13]. The prevalence of mutations in protein C (PC), protein S (PS) and antithrombin (AT) is 0.023, 0.037 and 0.019, respectively; despite the low prevalence, the congenital deficiencies of these natural anticoagulants are the most important inherited thrombophilic conditions. Newborns with severe deficiency of PC, PS, or AT (due to homozygous or compound heterozygous conditions) usually present with purpura fulminans or extensive multiple TEs. More recently, some studies indicated that increased lipoprotein(a) [Lp(a)] concentrations play an important role in childhood and neonatal thrombosis in the German population [14–16]; however it is unclear whether this can be applied to all populations. Other very rare disorders, such as dysfibrinogenemia, dys/hypoplasminogenemia and homozygous homocystinuria have been associated with an increased risk of thrombosis but their role in favoring thrombotic events in newborns is unclear.

The evaluation of a neonate with a significant TE event for a genetic prothrombotic disorder is a debatable subject. There is some evidence that an infant with multiple trait thrombophilia and symptomatic thrombosis is at increased risk of thrombus recurrence, although those recurrent events usually occur in the setting of other comorbidities, such as infection, surgery, or trauma. In addition, there are no recommendations on secondary prevention of thromboses in infants with thrombophilia. However, many experts believe that screening for thrombophilia is warranted in a neonate with a symptomatic TE, regardless of other acquired risk factors [17, 18].

100.4 Maternal Thrombophilia and Gestational Risk Factors

Few data are currently available regarding the influence of maternal thrombophilia (prothrombotic polymorphisms, dislipidemia, antiphospholipid antibodies) and prenatal gestational risks (gestational diabetes, pregnancy-induced hypertension-preeclampsia, intrauterine growth restriction) on thrombotic risk in the fetus and neonate.

During gestation, the maternal and fetal circulation systems coexist and interact with the placenta. Association between thrombosis and infarction in the placenta and peri- and neonatal infarction, the most commonly presumed mechanism being embolization to the fetus, has been reported in literature [19–21].

Pregnancy itself is a prothrombotic state as shift toward prothrombotic reactions is seen in women as gestation progresses through the second and third trimesters and just after gestation (increased levels of vitamin K-dependent FII, FVII and FX; increased levels of thrombin anti-thrombin (TAT), increased endothelial activation with elevated levels of circulating von Willebrand factor (VWF) and soluble thrombomodulin; increased triglyceride and Lp(a) levels; reduced levels of free and total PS and reduced activated PC sensitivity ratio) [22]. However, maternal thrombosis during and just after pregnancy often occurs in the presence of additional risk factors (i.e., advanced maternal age, cesarean section, hypertension, gestational diabetes, infections, pre-eclampsia/eclampsia, antiphospholipid syndrome, and inherited thrombophilia). Some of these maternal factors may also predispose to placental infarction with subsequent cerebral infarction in the fetus.

Pre-eclampsia may lead to endothelial activation of coagulation. Gestational diabetes leads to maternal vascular damage, which can predispose to placental thrombosis and infarction. In case of chorioamnionitis, inflamed vessels of the placenta lead to localized thrombosis, vasospasm and infarction and maternal sepsis during gestation may lead to disseminated intravascular coagulation (DIC) resulting in placental thrombosis and infarction, too. Maternal smoking may lead to vasospasm and/or endothelial activation with resulting placental injury and growth restriction and maternal cocaine use may predispose to vasoconstriction with resulting placental infarction. Moreover, thrombocytosis has been observed in neonates of mothers with history of polydrug use. At the time of delivery, placental-fetal transfusion may lead to polycythemia [23]. Antiphospholipid syndrome (APLS) in pregnancy is characterized by the presence of auto antibodies in association with recurrent fetal loss and severe complications such as pre-eclampsia, fetal growth restriction, or placental insufficiency [24].

In a review of the literature until 2007 of infants born to mothers with antiphospholipid antibodies (aPL), 16 infants with perinatal thrombosis were reported. Thromboses were arterial (13/16), mostly strokes (8/16). Hydrops fetalis with left renal vein thrombosis was associated with a lupus anticoagulant (LA) present only in the child. Additional risk factors to aPL, either prenatal (pre-eclampsia and/or intrauterine growth restriction) or perinatal (asphyxia, sepsis, arterial or venous catheter and congenital thrombophilia) were present (one to four of them) in nine out of the 14 evaluable babies. aPL were the only risk factor found in five full term babies who suffered from stroke in four cases and from renal thrombosis in another. Eleven of these infants with aPL in their serum presented a neonatal APS with the same antibody (LA or aCL IgG) found

in neonates and their mothers, while the other infants had thrombosis with aPL only in their mother's blood.

100.5 Environmental and Iatrogenic Modifications

Endothelial, coagulation, and fibrinolytic systems are activated in newborns; this might occur during birth due to the mechanical stress, the adaptation of circulation, and the short-term hypoxic state [25].

Generation and inhibition of thrombin was compared with healthy neonates. Fifty neonates with respiratory failure requiring mechanical ventilation and 40 healthy neonates were studied on day 1 of life. Eight plasma pools from 40 healthy neonates of GA 30 LA or aCL 38 wk were compared with six plasma pools from 30 sick neonates of GA 30-38 wk. An additional four plasma pools prepared from 20 sick neonates of GA less than 30 wk were studied. Thrombin generation was similar for both healthy and sick neonates of GA 30-38 wk. However, the inhibition of thrombin was impaired in plasma from sick neonates of GA 30-38 wk compared with plasma from healthy neonates of GA 30-38 wk (4.37 ± 0.22 versus 5.21 ± 0.21 nmol; p less than 0.05) [26].

A combined hemostatic defect consisting of a reduction in certain procoagulant, anticoagulants (AT, PC) and components of the fibrinolytic system (PLG) was demonstrated in very-low-birth-weight infants (VLBW <1,500 g) with gestational age (GA) 26–32 weeks. Sixteen of them were healthy, 28 were suffering from RDS and 24 from septicemia. The hemostatic defect was more profound in the RDS group, nevertheless increased TAT and/or PAP values were a more frequent finding in the septicemic group of infants (91.8 *vs* 17.9%). Increased D-Dimers were demonstrated in 34.8 and 28.6% of the infants, respectively [27].

The modality of delivery (spontaneous vaginal delivery *vs* elective caesarian section) has been demonstrated to be able to modify the levels of PS, PC and AT; plasma collected from cord blood of 41 consecutive healthy newborns, 18 delivered vaginally (mean GA 39.7 ± 0.8) and 23 by elective caesarian section (mean GA 38.5 ± 0.7) were tested for AT activity, PC antigen and activity, total and free PS antigen, F1 concentration and PLG activity; among factors studied in cord blood of infants born after vaginal delivery, PC antigen levels and AT activity were statistically higher (41.3 ± 9.4 *vs* 33.9 ± 7.2 and 58.5 ± 10.0 *vs* 48.4 ± 12.7, respectively; $P < 0.01$), while free PS was significantly lower (36.8 ± 11.6 *vs* 46.4 ± 12.5; $P < 0.05$) than in newborns delivered by caesarian section. Cord blood PLG and F1 were elevated in vaginally delivered neonates in comparison to those delivered by caesarian section, but the difference was not statistically significant [28].

In a small group of newborns with CVL, increased platelet activation and VWF binding to platelets has been significatively recorded [29].

100.6 Neonatal Non-CNS Thrombotic Events

100.6.1 Epidemiology and Thrombus Location

Neonatal symptomatic thrombosis (excluding stroke) is reported to occur in 2.4/1,000 admissions to neonatal intensive care units according to an international Registry from Canada, USA, and Europe; 64 among 97 (66%) registered events are venous thrombosis [6]. In a German prospective nation-wide two-year registry, incidence of clinically apparent neonatal TEs (including cerebral events) is 5.1/100,000 live births; 76% are venous events [5].

Both arterial and venous non-CNS thrombosis in the newborn may be located in several anatomic sites (i.e., venous events: deep vein thrombosis of the limbs, intrathoracic vessels, pulmonary embolism, renal veins, hepatic veins, superior and inferior caval veins, portal vein, right intracardiac thrombosis, mesenteric veins, retinal vein; arterial events: aorta, left intracardiac thrombosis, renal artery, mesenteric artery and limb artery).

100.6.1.1 Venous Systemic Thrombosis

Neonatal renal vein thrombosis (RVT) is the most common non-catheter-related thrombosis in infancy. It has been reported to occur in 0.5/1,000 admissions in neonatal intensive care units; according to the German Registry the incidence is 2.2/100,000 live births [30]. It usually presents within 3 days after birth in neonates born at term; in 7% of neonates it presents in uterus and a male predominance has been reported [31, 32].

Central venous line (CVL) related venous thromboses (VT) are commonly associated with indwelling central venous catheters. Compared to adults, newborns presents a higher incidence of thrombosis of the superior limbs, due to the strong association with CVL placement in the upper venous system [33].

Portal vein thrombosis (PVT) is described in the neonate secondary to umbilical venous catheter insertion. A retrospective analysis reported 133 infants (all but 5 of whom were neonates) with portal vein thrombosis, with an incidence of 3.6/1,000 admissions; an umbilical vein catheter had been inserted in 73% of the infants and was not in an appropriate position in half of them [34].

The frequency of pulmonary embolism (PE) during the newborn period is unknown but likely underestimated because the clinical features are often subtle or masked by the presence of underlying lung disease. In a retrospective autopsy series an incidence of 14% has been reported [35].

100.6.1.2 Arterial Systemic Thrombosis

Arterial thromboses (ArT) represent almost 50% of all neonatal TEs and are mainly iatrogenic complications, associated

with catheterization of the femoral artery as well as peripheral arteries and umbilical artery.

The reported incidence of umbilical artery catheter-related TE is considerably influenced by the diagnostic test chosen. Studies considering only symptomatic TEs report an incidence of 1–3%, while studies using ultrasound and angiography report an incidence of 14–35% and 64%, respectively. In autopsy series an incidence of 9–28% has been reported [36]. Spontaneous, non catheter related ArT are uncommon in the newborn period and most frequently involve the aorta. Mortality rate has been reported in around 33% of pediatric thrombotic disease [37].

100.6.1.3 Intracardiac Thrombosis

Venous intracardiac thrombosis involving the right atrium frequently occurs in newborns with central venous lines (CVL), but no incidence data are reported in the literature. Thrombosis involving the right ventriculum is uncommon. Left intracardiac thrombi (atrium and ventriculum) are rare events usually occurring in children with underlying risk factors such as congenital heart disease, cardiovascular surgery or arrhythmia [38–40].

100.7 Risk Factors

100.7.1 Inherited Thrombophilia

At present, the role of thrombophilic proteins in both arterial and venous systemic thrombotic events in the neonate remains poorly defined; on the other hand, the presence of an indwelling line is clearly identified as the most important risk factor.

Manco-Johnson et al [41] did not find any association between thrombophilia and catheter related events in newborns, in contrast with previously published data by Nowak Gottl et al [42] who detected an high incidence of inherited prothrombotic factors in a cohort of infants and children, ranging from neonates to 18 years, with catheter related events.

In the registry from Netherlands, a higher incidence of thrombophilia in older children (21%, 95% CI 8–34%) than in neonates (6%, 95% CI 0–16%) was detected [7], while in the study by Revel-Vilk et al [43] the overall frequency of inherited prothrombotic proteins was similar in newborns and older children (13%). Kosch et al [44] investigated the prevalence of prothrombotic risk factors in renal vein thrombosis: the study evidenced a significant association with genetic risks, especially FVL and Lp(a).

Registry data and case studies have demonstrated that the majority of symptomatic neonatal TEs, especially spontaneous events (i.e., not catheter-related) are associated with multiple hemostatic prothrombotic defects or with the combination of prothrombotic defects and environmental or clinical conditions. As a result, the Perinatal/Pediatric Hemostasis Subcommittee of the International Society on Thrombosis and Hemostasis has recommended that pediatric patients with thrombosis (regardless of risk factors) should be tested for a full panel of genetic prothrombotic traits [17].

100.7.2 Acquired Risk Factors

The presence of an indwelling line has been identified as the most important acquired risk factor for the development of both arterial and venous systemic events, spontaneous non catheter-related events being relatively uncommon. Neonates in the neonatal intensive care unit may have umbilical artery (UAC) and vein (UVC) catheters as well as additional intravenous lines. These can cause damage of the endothelium wall and activation of the coagulation cascade. In a large prospective study, 148 neonates with both umbilical arterial and/or venous catheters were serially screened for thrombosis in abdominal aorta and inferior vena cava by 2-D ultrasonography. Abdominal aortic thrombi were detected in 32.3% of infants with UAC and small thrombi were detected in the inferior vena cava of 4.1% of infants with UVC. The only significant risk factors associated with abdominal aortic thrombosis was the presence of UAC compared with UVC and longer duration of UAC in situ [45]. Excluding renal vein thrombosis, catheter-related events accounted for 89% and 94% of TEs in the Canadian and Netherlands' Registry, respectively [6;7].

Central line placement is a recognized risk factor also for thrombosis involving the right atrium. Other identified risks for intracardiac events are congenital heart diseases (i.e., aortic coarctation), cardiac surgery, and ECMO [39, 40, 46].

Perinatal asphyxia, prematurity, dehydratation and maternal diabetes are established risk factors for renal vein thrombosis. However, in a recent review by Lau et al [32], pertaining to all the available literature about venal rein thrombosis, among 271 patients less than one third had a history of perinatal asphyxia, and dehydratation and maternal diabetes were even less common. One third (28%) of patients were before 36 weeks of gestation.

Numerous clinical and environmental conditions such as the use of central lines, cardiac diseases and polycythemia, renal diseases such as congenital nephrotic syndrome and neonatal hemolytic uremic syndrome, peripartum asphyxia, infants of diabetic mothers, dehydration, septicemia, necrotizing enterocolitis, acute respiratory distress syndrome, and extracorporeal membrane oxygenation lead to elevated thrombin generation and subsequent thrombus formation [47].

100.8 Clinical Investigation

The clinical picture of vascular incidents in neonates is extremely variable and largely dependent on the location and

the size of the thrombus or embolus. Presentation may vary from discrete symptoms or asymptomatic events to life or limb threatening acute events; as several right-to-left (i.e., venoarterial) shunts remain patent for a considerable time in the neonate, a paradoxical embolus cannot be ruled out even in the absence of congenital heart disease.

100.8.1 Arterial Thrombosis

Arterial vascular events are almost always directly related to arterial vascular catheterization of central or peripheral arteries. This underscores the need for meticulous clinical observation in children with arterial access in place. Obvious signs include ischemia of limbs or trunk, pale or cold extremities distal to the catheterization site, weak or absent palpability of the pulse (pulse oximeters will also show this) and decreased or immeasurable blood pressure. Signs of necrotizing enterocolitis such as feed intolerance, bilious gastric aspirates, blood-stained stools, and bowel wall pneumatosis in an infant with an umbilical arterial catheter should raise the suspicion of mesenteric infarction. Diagnostic work-up for renal failure in children with UAC should include a Doppler flow study of the renal arteries to avoid missing out on the diagnosis of renal artery thrombosis. Elevated blood pressure might also hint at decreased renal perfusion.

There are some reports of aortic thrombosis in the neonate mimicking coarctation of the aorta with significant blood pressure differences in upper and lower body blood pressure readings; thus, the simple investigation of upper and lower body blood pressure measurements should be performed in any infant with hypertension diagnosed by measurements obtained from the upper body [48].

100.8.2 Venous Thrombosis

Limb swelling, pain, and cyanotic or hyperemic color should raise the suspicion of venous thrombosis.

100.8.2.1 Renal Vein Thrombosis

RVT may present with an abdominal mass and hematuria or proteinuria; hence, these symptoms should ring a bell especially in neonates with risk factors such as prematurity, asphyxia, critical illness, femoral CVL, and male sex [30, 31].

100.8.2.2 Portal Vein Thrombosis

Signs of impaired liver function, hepatomegaly and splenomegaly should raise the suspicion of PVT; however, only about 10% of children with PVT develop acute clinical symptoms [34]. Thrombosis of the inferior vena cava can present with signs resembling obstruction of the renal vein (hematuria, retroperitoneal mass); however, these will occur bilaterally when the inferior vena cava is affected. In addition, the lower limbs may be edematous and, if blood flow is substantially impaired, the child may be in respiratory distress and may have high blood pressure.

100.8.3 Pulmonary Embolism

PE is a rare event in the neonatal period. Clinical signs of massive ventilation/perfusion mismatch, difficult oxygenation, and signs of right heart failure should raise the suspicion of PE, especially in babies with congenital heart disease. In the few cases reported in the literature, diagnosis of PE was confirmed by lung perfusion scans or angiography [49–51].

For further information on central nervous system thrombotic events, see Chapters 139 and 142.

100.8.4 Laboratory Investigation in Newborns with Thrombosis

Initial laboratory work-up in a neonate in whom thrombosis is suspected should include a full blood count as well as a coagulation screening with determination of prothrombin time, thrombin time and activated partial thromboplastin time.

100.8.4.1 D-Dimers

D-Dimers, which are elevated as an acute phase reaction in all patients with infection or a systemic inflammatory response syndrome, are a positive finding in almost all critically ill neonates. Conversely, negative D-Dimers might be relatively accurate in ruling out thrombosis in most patients, including neonates.

100.8.4.2 Platelets

In almost all neonates, platelets number decreases after birth. However, a sudden and severe drop in platelet counts should alert the intensivist. Although the differential diagnosis for thrombocytopenia in the neonate is broad, including auto- and allo-antibodies as well as effects of medications and consumption, thrombocytopenia remains one of the most sensitive indicators for micro- (in the setting of sepsis) or macro-circulatory thrombosis.

100.8.4.3 Thrombophilia Testing

Current evidence does not resolve the issue of thrombophilia testing, but a stepwise thrombophilia investigation in any

Table 100.1 Thrombophilia testing

Initial Laboratory Testing
Antiphospholipid antibody panel
Anticardiolipin antibodies and lupus anticoagulant (IgG, IgM)
Fibrinogen
Protein C activity
Protein S activity
Antithrombin activity assay
Factor V Leiden
Prothrombin G20210A
MTHFR C677T
Homocysteine
Lipoprotein(a)
Additional Laboratory Testing
Factor VIII activity
Factor IX activity
Factor XI activity
Plasminogen activity
Heparin cofactor II activity

neonate with a significant TE event is reasonable. A limitation to this approach is the amount of blood required. This is especially important in the premature or anemic infant, for whom excessive blood loss is not tolerable. Therefore, the evaluation should always take place at an experienced tertiary care center that has either a reference laboratory or a reliable referral center. The physiological reduction of some coagulation factors and anticoagulant proteins may lead to a difficult interpretation of laboratory investigations and therefore deficiencies need to be confirmed by repeat testing later in infancy (i.e., beyond 6 months or afterward in premature infants) [18]. As screening all newborns with TEs for a wide range of prothrombotic disorders may be prohibitively expensive, targeted screening may be more effective.

In case of a clinically significant spontaneous TE or ischemic skin lesions, it is mandatory to look for homozygous deficiency of PC and PS, as a specific treatment option is available. In remaining cases a stepwise approach starting with most common risk factors may be reasonable. Testing of natural anticoagulants for detecting a heterozygous defect should be performed after 6 months of age. The protein-based assays are affected by the TE event and must be repeated at 3–6 months of life, before a definitive diagnosis can be made. If anticoagulation is being administered, then the protein-based assays should be obtained 14–30 days after discontinuing the anticoagulant.

DNA-based assays are neither influenced by the acute thrombotic event nor by the treatment; Lp(a) concentrations tend to increase during the first year of life and should be repeated at 8–12 months if the original values are low, especially in white individuals.

In case of positive result for genetic thrombophilia in the newborn, testing of both parents (FVL and PRT G20210A mutations, Lp(a), natural anticoagulants) may be offered; in case of negative screening, testing of mother has been suggested for the newborn with perinatal stroke [52]. All these tests should be performed in the non acute setting as they are not useful to support management decisions but only for counseling about possible risks to parents theirselves and of other or future children. However, in those countries where the health system is mainly managed by insurance companies, family members should be carefully informed about the possible consequences of this screening (i.e., potential insurance issues) without potential specific benefit to the individual being screened.

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Coagulation Disorders: Inflammation and Thrombosis

Jennifer L. Armstrong-Wells and Marilyn J. Manco-Johnson

101.1 Introduction

With a fifteen-fold increase in rate compared with infants and children, thrombosis is an important problem in the neonate. Most neonatal thromboses are diagnosed in the intensive care nursery in association with myriad underlying disorders, many of which are inflammatory. Clinically, most cases of neonatal thrombosis occur in two discrete populations: spontaneous perinatal events (primarily stroke and renal vein thrombosis) or catheter-related thrombosis related to neonatal intensive supportive care. The relationship of inflammation to these two categories of thrombosis is currently unknown.

A bidirectional cross-talk between inflammation and thrombosis has been elucidated in recent years [1]. Inflammatory cytokines activate coagulation via stimulation of tissue factor expression on endothelial and monocyte cell surfaces, decrease natural anticoagulant mechanisms and impair fibrinolysis [1]. The procoagulant enzyme thrombin activates the acute inflammatory response via intracellular signal transduction initiated through protease activated receptors (PARs) of endothelial cells [2]. The link between inflammation and arterial thrombosis and atherosclerosis has been clearly established in adults [3]. Data implicating inflammation and venous thrombosis has been less clear [4]. While the inflammatory marker C-reactive protein (CRP) performs similarly to D-dimer as a predictor in the diagnosis of venous thromboembolism (VTE) in adults, the use of CRP as a biomarker predicting the development of new VTE was not significant after correcting for the effect of body mass index (BMI); however, this risk factor is not clinically relevant in the newborn infant [4]. This chapter will explore evidence for the association of inflammation and thrombosis in perinatal arterial and venous events.

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101.2 Etiology and Pathogenesis

101.2.1 Placental Inflammation, Thrombi and Infarcts

A role for inflammation, coagulation activation and thrombosis in diverse fetal and perinatal pathologies was suggested by the finding of placental thromboses and infarcts in cases of fetal growth restriction or demise. Histologic evidence of chronic inflammation of the distal placental villous tree is found in 76–136/1000 live births; evidence for an infectious etiology of this placental inflammation, known as villitis of unknown etiology (VUE) has been lacking [5]. At the more extreme end of this pathologic spectrum, a true fetal large vessel vasculitis develops with vessel wall damage, occlusion and, potentially, release of inflammatory cytokines into the fetal circulation [5]. Fetal thrombotic vasculopathy is significantly more frequent in the placentas of term infants with neurologic impairment [6]. A systematic review of inflammatory biomarkers in term infants with neonatal encephalopathy determined elevated levels of interleukin-6 (IL-6) in cord blood as positive predictors of adverse neurologic outcome [7]. Whether neurologic damage in fetal thrombotic vasculopathy is mediated by thrombotic ischemia or direct inflammatory damage to neurons is currently unresolved.

101.2.2 Mutations in Genes Involved in Inflammation

Reports of vascular impairments in infants found to carry genetic mutations affecting proteins involved in inflammation have shed rare clues into the physiologic gestational and perinatal interactions between inflammation and coagulation. A polymorphism in the promoter region of the cyclooxygenase-2 gene (-765 G>C), conveying 30% reduction

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

in the expression of COX-2 levels with GC or CC polymorphisms compared to GG, was associated with a three-fold reduction in histopathological evidence of placental ischemia and malperfusion and a four-fold reduction in the rate of intrauterine growth restriction [8]. Reduced levels of COX-2 have been associated with decreased levels of CRP and thromboxane A2 and increased prostaglandin E2 [9–11]. Conversely, an autoinflammatory disease caused by a 175-kb homozygous deletion at chromosome 2q13, which encompasses several interleukin-1 (IL-1) family members including the IL-1-receptor antagonist, was associated with marked elevations in markers of inflammation including CRP and extensive systemic thrombosis during the neonatal period [12].

In a study of over 1000 very low birth weight infants, there was no increase in adverse outcomes, including sepsis, bronchopulmonary dysplasia, intraventricular hemorrhage or periventricular leukomalacia in carriers of the thrombophilia mutations, factor V Leiden or Prothrombin 20210 [13]. Interestingly, the factor XIII-Val34Leu mutation, which results in thinner fibrin fibrils that are more resistant to fibrinolysis, was associated with an increased risk of sepsis. Additionally, the factor VII-323del/ins (323 A1/A2) promoter polymorphism, which is associated with a 20% decrease in plasma factor VII activity, was associated with a reduced risk of bronchopulmonary dysplasia. The tissue factor/factor VII complex promotes inflammation through activation of PARs; lower levels of factor VII activity could result in decreased inflammatory signaling. In both of these examples, mutations in coagulation genes exerted effects on neonatal outcomes through interactions with inflammatory pathways, rather than by direct effects on hemostasis and thrombosis.

101.2.3 Maternal Inflammation and Predictors of Fetal/Neonatal Thrombosis

Most investigations of neonatal inflammation and thrombosis have been performed in the context of arterial ischemic stroke. Perinatal stroke can be seen in a variety of systemic disturbances, and manifests as perinatal arterial ischemic stroke (PAS), perinatal hemorrhagic stroke (PHS), or cerebral sinovenous thrombosis (CSVT). While birth trauma and delivery-related anoxia were previously thought to be major causes of perinatal stroke, new studies now invoke other pathogeneses, including inflammation. Maternal factors, such as infertility, diabetes, pre-eclampsia and chorioamnionitis, have been associated with PAS [14–16]. Chorioamnionitis has also been associated with neonatal CSVT [17]. Further, fetal distress and postmaturity are strong predictors for intracerebral hemorrhage in term and late preterm newborns [18]. Therefore, inflammatory risk factors for perinatal stroke are identifiable before birth, allowing the unique opportunity for early screening and intervention for those newborns identified at risk.

101.2.4 Antiphospholipid Antibodies in Pregnancy

Maternal inflammatory factors may also play a pivotal role in neonatal thrombosis, including stroke, as pregnant women with antiphospholipid antibodies (APA) have a higher rate of placental thrombosis [19]. APA can be acquired and transiently observed with infection, tissue injury, and certain drugs, or can be part of an autoimmune syndrome. The diagnosis of obstetric antiphospholipid syndrome (APS) requires at lease one clinical and one laboratory criterion. Clinical criteria include objectively confirmed vascular thrombosis or pregnancy morbidity. Qualifying obstetrical complications include: three or more unexplained consecutive spontaneous abortions before the tenth week of gestation; one or more unexplained deaths of a morphologically normal fetal beyond the tenth week of gestation, with morphology confirmed by direct examination or ultrasound; or one or more births of a morphologically normal premature infant of less than 34 weeks gestation due to eclampsia, severe preeclampsia or recognized features of placental insufficiency [20].

It has been speculated but not proven that the syndrome of hemolytic anemia, elevated liver enzymes and low platelets (HELLP) of pregnancy is induced or exacerbated by APS in pregnancy. Laboratory criteria required for the diagnosis of APS include medium to high-titer IgG and/or IgM ACA or anti-β_2GPI antibodies detected in an ELISA assay or the presence of the lupus anticoagulant (LA). The diagnosis of the LA requires prolongation of a phospholipid-based clotting assay, failure to correct the prolongation with addition of normal plasma and correction with addition of excess phospholipid.

The mechanisms of adverse pregnancy outcomes in obstetrical APS include: direct fetal resorption and placental insufficiency; abnormal development of spiral arteries of the placenta; damage to placental trophoblasts; interference with the activation of protein C; interference in fibrinolysis; alterations in the structure and function of annexin V; increase in proinflammatory cytokines; and increase in complement activation [20]. Obstetrical morbidity and fetal loss increase with the number of positive APA tests [21]. Transplacentally-derived maternal APA may be risk factors for perinatal stroke or thrombosis [16, 22, 23].

101.2.5 Cytokines

Cytokines are small, soluble polypeptides that bind to cell surface receptors, instigating myriad responses by changing the behavior of target cells. Cytokines both mediate inflammation and are elicited as part of an immune response [24]. Individual cytokines rarely work in isolation, but rather act with other cytokines, whether it be by inhibition, synergism, or other unpredictable effects; they may also induce release of other cytokines, the so-called cytokine cascade [24].

Cytokines have recently been recognized as integral in endothelial cell maintenance. Cytokines can promote expression of adhesion molecules, while activating the endothelium, leading to procoagulant activity. Specifically, IL-1 has been shown to cause endothelial cells to generate and express tissue factor on their cell surface, which allows binding of Factor VIIa, and therefore initiates the extrinsic clotting pathway. IL-1 and tumor necrosis factor (TNF-α) also inhibit anticoagulant APC by decreasing thrombomodulin expression. Both IL-1 and TNF-α also increase plasminogen activator inhibitor type 1 (PAI-1), which inhibits the intrinsic fibrinolytic system of the endothelial cell. Therefore, IL-1 and TNF-α are thought to work in an additive fashion as procoagulant cytokines. Further, they are also classified as inflammatory cytokines, with ultimate induction of leukocyte activation and adhesion by the cytokine cascade (IL-1) as well as direct endothelial action and lymphocytic inflammation (TNF-α). Other cytokines have also been classified as proinflammatory, including IL-2, IL-6, and IL-8. Additionally, IL-18 (formally known as IL-1γ because of its homology to IL-1β) has been implicated in ongoing inflammatory brain injury [25, 26].

Because of the similarity between endothelial damage caused by inflammatory cytokines and damage determined in preeclampsia, levels of circulating cytokines in preeclampic women have been studied. Increased levels of TNF-α and IL-6 have been found to be increased in women with preeclampsia [27, 28]. It has been shown that in infants with neonatal hypoxia, encephalopathy or subsequent cerebral palsy, elevated serum levels of IL-1β, IL-6 or IL-8, cerebrospinal fluid levels of IL-1β or neuron-specific enolase, or brain concentrations of IL-1β or TNF-α have been determined [29, 30]. These proinflammatory cytokines, triggered by infection or hypoxia, are involved in a final common pathway within a molecular cascade that may lead to perinatal brain damage [31, 32]. Consequently, this inflammatory reaction is suggested to arise from the fetus itself – the so-called fetal inflammatory response – and has been postulated as a mechanism for perinatal brain injury [31]. Studies conducted to date demonstrate that the fetal inflammatory response and cytokine activation – with possible underlying genetic susceptibility – is associated with cerebral palsy in the term/late preterm newborn, and therefore may be also be associated in the pathophysiology of perinatal stroke [29–31].

101.3 Inflammation and Systemic Neonatal Thrombosis

Less data exist regarding the relationship of inflammation to thrombosis in other syndromes of neonatal thrombosis. In one study of newborn infants undergoing palliative surgery for single ventricle congenital heart disease, pre-operative elevations in CRP predicted post-operative venous thrombosis [32]. Thornburg and colleagues reported a positive association between thromboses and infections in newborn infants who did not have lines removed for infection and described a subset of catheter-related thromboses in newborn infants characterized by onset late in the presence of catheter associated blood stream infection [33]. They hypothesized that catheter-related thrombosis in the newborn infant may be caused by inflammation induced by infection. Other systemic thrombi that occur in the perinatal period, particularly renal vein thrombosis, could be hypothesized to occur through similar mechanisms of placental inflammation with systemic emboli through the umbilical vein; however, there is currently no objective evidence to support this.

101.3.1 Clinical Aspects

To date, there is almost no clinical application of existing data on the contributions of inflammation to the prediction, prevention or management of thrombosis in the newborn infant. The one exception is obstetrical APS in which clinical testing and management of the newborn infant by a skilled perinatal team can be facilitated by known APA positivity in the mother. There are some intriguing data that neonatal inflammation may be predicted by maternal obstetrical conditions, and that adverse consequences of fetal and neonatal inflammation, primarily effects on perinatal brain development, may be ameliorated by interventions targeted to antagonize neonatal proinflammatory cytokines and promote anti-inflammatory cytokines [31].

101.3.2 Differential Diagnosis

There is no direct application of emerging knowledge regarding placental and fetal inflammation on differential diagnosis of neonatal thrombosis. However, neonatologists caring for infants born to mothers with the obstetrical conditions discussed above that are characterized by the inflammatory response, may be alerted to a potential increased risk for thrombosis in these infants, facilitating prompt recognition and diagnosis of clinical thrombosis.

101.3.3 Prognosis

There is evidence from preliminary research studies that serum and cerebrospinal fluid IL-1β, serum IL-6 and cerebrospinal fluid neuron-specific enolase predict poor neonatal outcomes [29]. However, none of the research in cytokine biomarkers has been developed to clinical usage. The prognosis of neonatal thrombosis related to maternal or fetal/neonatal inflammatory conditions, such as choramnionitis or obstetrical APS, is related to the site, extent and response to therapy of the thrombus itself.

101.3.4 Therapy and Treatments

Currently, the only neonatal antithrombotic therapy specifically influenced by inflammation is neonatal thrombosis in an infant with APA. In addition to standard anticoagulant and thrombolytic agents, some affected infants have been treated with isovolemic exchange transfusion and low dose aspirin [23].

Recommendations for antithrombotic therapy in the newborn infant with thrombosis (excluding PAS) are given in Table 101.1 [34]. The risk of thrombus progression and recurrence in the newborn infant appears to be low. This may be because most perinatal inflammation promoting thrombosis appears to derive from placental and maternal inflammatory conditions that are resolved once the infant is born. For this reason, antithrombotic therapy in PAS is generally not indicated. Peripheral arterial thrombi may be treated until blood flow is restored and additionally for a brief period (e.g., 48 hours) thereafter; the entire course is often 10–14 days. The Chest guidelines for antithrombotic therapy suggest a standard duration of anticoagulation of three months for the neonate, equal to that recommended for older children and adults [35]. However, resolution of venous thrombi in the newborn infant is often rapid and thrombus recurrence risk is low. If the underlying trigger for thromboembolism is reversed, extracranial venous thrombi may be treated until thrombus resolution, which often occurs within 10–30 days. Neonatal renal vein thrombosis has been a particularly challenging lesion with no therapeutic regimen particularly effective and the majority of affected infants suffering sequelae of partial or complete renal infarction or hypertension [36, 37]. Although some advocate for the use of heparin-based therapy in neonatal CSVT, most clinicians agree that treatment is determined on a case-by-case basis; treatment may be withheld unless the thrombus is progressive.

Tissue plasminogen activator (TPA), interventional thrombolysis and/or mechanical thrombectomy is useful in selected cases of extracranial thrombosis, provided contra-indications are strictly followed as outlined in Table 101.2. Thrombolysis is indicated chiefly when life-or limb-threatening ischemia is present, and should be considered for completely occlusive thrombi of the aorta, superior or inferior vena cava, abdominal thrombi, and arterial occlusions of the limb. There is no role at this time for TPA, interventional thrombolysis or mechanical thrombectomy for cerebral thrombosis (PAS or CSVT) in the neonate.

Table 101.1 Dosing for antithrombotic therapy in children

	Unfractionated Heparin Continuous IV	Enoxaparin Every 12 hours subcutaneous	Tissue Plasminogen Activator Continuous IV
Loading dose	Newborn < 37 weeks: 50 U/kg Newborn ≥ 37 weeks: 100 U/kg	None	None
Initial maintenance dose	Newborn < 37 weeks: 20–25 U/kg/hr Newborn ≥ 37 weeks: 28 U/kg/hr (may need up to 50 U/kg/hr hr to achieve therapeutic anti-Xa level)	1.5 mg/kg/dose 1.5 mg/kg/dose (may require up to 2.0 mg/kg/dose)	0.06–0.12 mg/kg/hr 0.06–0.12 mg/kg/hr
Monitoring	Anti-Xa activity following 4–6 hours of infusion; Target range 0.3–0.7 U/mL	Anti-Xa activity 4 hours following 1st or 2nd dose; Target range 0.5–1.0 U/mL	

Table 101.2 Contraindications to specific antithrombotic therapies in newborn infants and children

Unfractionated heparin	Low molecular weight heparin	Systemic TPA	Thrombolysis by interventional radiology
Known allergy	Known allergy	Known allergy	Known allergy
History of HIT(T)s	Invasive procedure < 24 hours	Active bleeding	In cases where needed, inability to place a Greenfield filter
		CNS ischemia/Surgery ≤ 10 days (includes birth asphyxia)	Limitations: Size of involved vessels and experience of interventionalists
		Surgery ≤ 7 days	
		Invasive procedure ≤ 3 days	
		Seizures ≤ 48 hours	
Fibrinogen < 100 mg/dL * Platelet count < 50,000/μL *	Fibrinogen < 100 mg/dL * Platelet count < 50,000/μL *	Fibrinogen < 100 mg/dL * Platelet count < 50,000/μL * INR > 2 *	Fibrinogen < 100 mg/dL * Platelet count < 50,000/μL * INR > 2 *

* With transfusion support, if necessary.

101.4 Conclusions

Evidence for inflammation as a cause of neonatal thrombosis exists mainly in studies of placental pathology and perinatal arterial ischemic stroke, but data relating catheter thrombosis to infection and inflammation is intriguing. Future research directed at determination of biomarkers for maternal inflammation may allow us to predict and prevent thrombosis in the developing fetus and newborn infant. The application of anti-inflammatory therapies, directed at activations of cytokines, complement proteins and inflammatory cells, applied to decrease cellular dysfunction and tissue infarction consequent to inflammation and thrombosis are in early stages. Anti-inflammatory therapies offer the hope of substantial advancement of therapy, particularly for perinatal arterial ischemic stroke.

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Coagulation Disorders: Clinical Aspects of Platelet Disorders

Antonio Del Vecchio

102.1 Introduction

Platelets contribute to primary hemostasis and their function is affected by their number and condition. Platelets arise from the fragmentation of megakaryocytes in the bone marrow, and circulate in the blood as disk-shaped non-nucleated particles, with a lifespan of 7–10 days. Their average diameter is about 1.5 μm, 20% of the diameter of erythrocytes. Once released from the bone marrow, young platelets enter the circulation, where a large proportion pool in the spleen. It has been suggested that this splenic sequestration of young platelets is the result of a longer transit time of large platelets through the splenic cords. The spleen serves as a reservoir for platelets, containing about one-third, which are then able to enter the circulation after exercise or epinephrine administration. In addition to the spleen, the lungs also contain a small pool of platelets, perhaps 10–15% of the total number, and these are also able to enter the circulation after exercise or epinephrine administration [1].

Blood platelets adhere to sites of vascular injury, generate biological mediators, secrete their granule contents, form multicellular aggregates and serve as a nucleus for plasma coagulation reactions. When endothelial continuity is disrupted, platelets adhere to the exposed basement membrane collagen and change their shape from smooth disks to spiny spheres, interacting with subendothelium-bound von Willebrand factor (vWf) via the membrane glycoprotein Ib (GPIb) complex (platelet adhesion). Subsequently, they secrete and release proaggregatory substances, such as adenosine 5'-diphosphate (ADP) and they synthesize thromboxane A2 from arachidonic acid. Additional platelets are recruited and form aggregates on those platelets that are adherent to the vessel wall to consolidate the initial hemostatic plug. A platelet glycoprotein IIb/IIIa (GPIIb-IIIa) complex mediates platelet-to-platelet interactions (platelet aggregation), a primary hemostatic plug is formed and bleeding is stopped (primary hemostasis).

Platelets also provide an extensive phospholipid surface for the interaction and activation of clotting factors in the coagulation cascade. Enzymes and cofactors of the coagulation system, and a fibrin mesh further stabilise the initial hemostatic plug (secondary hemostasis). Collectively, they help to maintain the integrity of the vascular system [2].

Disorders of platelets produce defects in primary hemostasis and result in signs and symptoms which differ from those of coagulation factor deficiencies. The usual clinical presentation of a primary hemostatic disorder is that of mucosal bleeding, epistaxis, petechiae, and purpura. By contrast, defects in secondary hemostasis show delayed deep tissue bleeding into muscles and joints.

102.2 Platelet Function in Neonates

The hemostatic system of neonates matures during the early weeks and months of life. It has been well characterised with regard to plasma coagulation factors. However, because of the difficulty of obtaining adequate volumes of blood for aggregation tests, few data are available about the function of neonatal platelets. GPIb appears on platelets early during fetal life and is present in fetuses of 18–26 weeks' gestation in greater amounts than those found in adults. The plasma of neonates contains higher concentrations of vWf than adult plasma, with an increased proportion of functional high-molecular-weight forms, which result in enhanced platelet adhesion. In the immediate newborn period, this enhanced platelet adhesion may compensate for the decreased intrinsic platelet activation in healthy neonates, but may leave sick neonates at increased risk of bleeding.

Platelets also express GPIIb-IIIa early in gestation, and at 27 weeks' gestation fibrinogen is found in concentrations similar to those in adults with normal binding to newborn platelets. Studies of platelet adhesion and platelet aggregation

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

evaluating the responses to agonists are not easily performed in newborn infants, and frequently generate conflicting results. There is diminished platelet function in neonates, as measured by aggregation in response to a number of physiological agonists [1, 3]. Recent studies, using flow cytometry of cord blood and peripheral blood of neonates, demonstrated that platelets from term and preterm neonates display generalized hyporeactivity to thrombin, collagen, ADP, and U46619 (a stable thromboxane A2 analog) during the first few days of life. Platelet responses reach normal adult levels between the fifth and the ninth days of life. Persistence of platelet hyporeactivity in neonates after the tenth day of life may therefore suggest a platelet disorder.

The mechanism for the decreased reactivity of neonatal platelets has not been adequately explained. Platelet hyporeactivity was formerly considered to be the result of platelet activation and degranulation during labor and delivery, but more recent studies of platelet activation markers have not supported this theory [4]. Decreased activation is due to relative deficiencies of phospholipid metabolism, calcium mobilization, granule secretion, and aggregation. Although the hyporesponsiveness of neonatal platelets depends on gestational age, and is indicative of the maturation of the hemostatic system, this transient dysfunction may be a developmental phenomenon to protect the neonate from the possible harmful effects of birth stress on the coagulation system [1].

102.3 Clinical Aspects

Compared with adults or older children, the hemostatic system of neonates is naturally immature with a low functional reserve capacity. In spite of this, neonatal hemostasis is generally in equilibrium, and there is a relatively low incidence of inappropriate bleeding in term infants [1–3]. However, in the presence of additional risk factors such as prematurity, asphyxia or infection, the neonatal hemostatic system is easily overwhelmed and bleeding can be rapid and dramatic.

Abnormalities of platelet function or of platelet-vessel wall interactions should be considered, particularly in infants who have platelet-type bleeding (e.g., petechiae, purpura, mucous membrane bleeding) with a normal platelet count [1]. A precise understanding of the type of platelet dysfunction and identification of the individual disorder allows for appropriate therapy.

102.4 Quantitative Disorders of Platelets

102.4.1 Thrombocytosis

A moderately elevated platelet count in the range of 450,000/μL to 600,000/μL is not an uncommon finding in the neonatal population, although a platelet count peak above 600,000/μL is rare. No adverse outcomes related to thrombocytosis have been described. In many preterm infants, a moderately increased platelet count has been observed at around 4–6 weeks of age, at the same time as the nadir of anemia of prematurity [5, 6].

Initial studies on the administration of recombinant erythropoietin (rEPO) to preterm infants reported an increase in hematocrit and reticulocyte numbers, followed by a period of iron deficiency. This iron deficiency and decreased ferritin levels was accompanied by a significant thrombocytosis. Iron deficiency is known to cause thrombocytosis in older children [7], but this relationship was not noticed in NICU patients before the introduction of rEPO treatment.

A secondary reactive thrombocytosis has been described in very young infants, particularly during infection. Antimicrobial therapy can also cause thrombocytosis in neonates treated with ceftriaxone, aztreonam, imipenem-cilastatin, ceftizoxime, and ceftazidime for suspected infection. No morbidity is generally associated with the elevated platelet count, and no treatment is necessary [6].

Early elevation of serum thrombopoietin levels is also related to subsequent thrombocytosis in low-birth-weight preterm infants [8]. Infants with Down syndrome occasionally have a slightly raised platelet count between 6 weeks to around 1 year of age, and thrombocytosis may occur in infants with congenital adrenal hyperplasia (CAH) [9]. The latter may have a normal hemoglobin, hematocrit and red blood cell counts while having significantly elevated white blood cell and platelet counts, possibly due to a stress-related response by marrow precursors as may occur in the course of sepsis. Usually, thrombocytosis resolves with the successful treatment of CAH [10]. Thrombocytosis is also seen occasionally in neonates with gastroesophageal reflux disease and following maternal use of methadone, but the causes and significance of these varieties of thrombocytosis are unclear.

102.4.2 Thrombocytopenia

Thrombocytopenia is one of the commonest hematological problems in neonates, affecting 22–35% of infants admitted to the neonatal intensive care unit, and almost 50% of all sick preterm neonates. The incidence of neonatal thrombocytopenia varies greatly, depending on the patient population and on the definition of thrombocytopenia. The platelet count in healthy neonates is similar to the range observed in older children and adults (150,000/μL to 450,000/μL), even though various centers, mostly in Europe, consider 100,000/μL as the lowest limit. Premature infants may have slightly lower values than term infants, since fetal platelet counts increase linearly with gestation. Recent data suggest a lower normal limit of 120,000/μL for preterm neonates during the first days following birth [11]. Thrombocytopenia is defined as mild when platelet count is

between 100,000/μL and 150,000/μL, moderate between 50,000/μL and 100,000/μL, and severe < 50,000/μL.

One classification of thrombocytopenia is based on the timing of onset: early (≤ 72 hours of life) *vs* late (> 72 hours). Early thrombocytopenia is commonly associated with feto-maternal conditions, reduced platelet production being secondary to placental insufficiency and fetal hypoxia, intrauterine growth restriction or maternal hypertension. The resulting neonatal thrombocytopenia is usually mild to moderate, resolves spontaneously and does not generally warrant treatment. The most frequent causes of thrombocytopenia occurring after the first 72 hours of life, both in preterm and term infants, are sepsis and necrotising enterocolitis and the associated thrombocytopenia can be severe and protracted, often requiring therapy [12].

Variation from this pattern of thrombocytopenia suggests an immune thrombocytopenia with an increased risk of hemorrhage, since in this case the thrombocytopenia is usually severe (platelets <50,000/μL). Thrombocytopenia in the neonate can be asymptomatic or can present with a variety of symptoms, including petechiae, melena, hematuria, blood-stained endotracheal secretions, and bleeding from previous puncture sites. Other clinical signs of thrombocytopenia are intraventricular hemorrhage (IVH) or pulmonary hemorrhage [13].

An infant with clinical signs of thrombocytopenia but without coagulation abnormalities should prompt examination for hepatosplenomegaly, congenital anomalies, and/or signs of sepsis.

In the mother, the platelet count, appearance of the blood smear, drug history, and evidence of transmissible infection should be investigated. Parental consanguinity and/or hematologic disease in family members should also be excluded [14]. Neonatal thrombocytopenia is broadly due to the following mechanisms: decreased platelet production, increased platelet destruction, or platelet sequestration [15].

102.4.2.1 Thrombocytopenia Due to Decreased Platelet Production

Overlap exists between the varieties of thrombocytopenia among neonates with intrauterine growth restriction (IUGR) and those with pregnancy-induced hypertension (PIH). Placental insufficiency and/or fetal hypoxia are likely involved in the reduced production of platelets [16]. Plasma concentrations of thrombopoietin are generally elevated in these patients, who have low to normal platelet counts (150,000/μL to 200,000/μL) or mild thrombocytopenia (100,000/μL to 150,000/μL) in the first day after delivery. Thereafter the platelet count usually falls, with a nadir on days 4–5, generally recovering to above 150,000/μL by days 7–10. If not complicated, the nadir is rarely below 50,000/μL, and the risk of hemorrhage is not significant. These patients usually do not need platelet transfusions or other treatments for thrombocytopenia [13].

Most cases of decreased platelet production are the consequence of reduced megakaryocytes or impaired platelet production by megakaryocytes. Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare disorder with absence or near absence of megakaryocytes in the bone marrow, and a risk of developing aplastic anemia. Compound heterozygosity for two-chain termination point mutations in the Mpl gene (thrombopoietinreceptor) has been identified. Consequently, plasma thrombopoietin levels in these patients are significantly elevated. Both X-linked and autosomal recessive patterns of inheritance have been reported. Bone marrow transplant is currently the only treatment [17, 18].

Thromobcytopenia with absent radii syndrome (TAR) is an autosomal recessive disorder with hypomegakaryocytic thrombocytopenia and bilateral absence of the radii, with the presence of functional thumbs. Bone marrow samples show decreased, absent, or immature megakaryocytes. No thrombopoietin gene mutations have been found. TAR may be associated with congenital heart disease, renal problems, defects of the lower limbs, and other hematological abnormalities, including aleukemoid reaction, eosinophilia and anemia. There is symptomatic thrombocytopenia within the first 4 months of life in 90% of patients, often resulting in gastrointestinal or intracranial bleeding. The elevated level of thrombopoietin despite normal Mpl suggests that the imperfect thrombocytopoiesis in TAR is probably due to a lack of response to thrombopoietin in the signal transduction pathway [17].

Fanconi anemia is an autosomal recessive disorder associated with skeletal and genitourinary abnormalities. These patients can become pancytopenic. It is important to note that 25% of affected patients are structurally normal and the diagnosis should be considered in presence of thrombocytopenia, anemia, leukopenia, in the absence of dysmorphic features.

Thrombocytopenia associated with a myeloproliferative disorder or with a wide variety of hemostatic abnormalities has been reported in Noonan syndrome, a rare autosomal dominant disorder with facial anomalies, congenital heart disease, skeletal abnormalities, and genital malformations [19]. Alport's syndrome, a genetic disorder associated with nephritis and nerve deafness, may present with thrombocytopenia and macrothrombocytes. Wiskott-Aldrich syndrome is a rare, sex-linked recessive disorder with thrombocytopenia, eczema, a tendency to severe bleeding and an increased incidence of recurrent infections because of an impairment of cellular and humoral immunity. There is also an increased incidence of malignancy, such as lymphoma. The platelets are small, with one-half normal volume and decreased platelet-mass, and their aggregation and life span also is diminished. The general prognosis is poor, and marrow transplantation is considered the treatment of choice [14].

Thrombocytopenia is also present in other chromosomal abnormalities. For instance, thrombocytopenia is found in 87% of infants with trisomy 18, in 54% of those with trisomy 13, in 31% of those with Turner's syndrome, and in 28% of those with trisomy 21 [1].

102.4.2.2 Thrombocytopenia Resulting from Increased Platelet Destruction

Immune Thrombocytopenias

Autoantibodies, alloantibodies, or drug-dependent antibodies may combine with platelet membranes and produce platelet impairment, resulting in platelet removal by phagocytes of the reticuloendothelial system. Immune destruction of platelets may be prompted by antibodies against a platelet membrane antigen, or antibodies as part of an immune complex that binds Fc receptors on platelets. These immunologic mechanisms are responsible for various clinical syndromes, including neonatal alloimmune thrombocytopenia (NAIT), autoimmune thrombocytopenia and post-transfusion purpura.

NAIT is the platelet counterpart of hemolytic disease of the neonate. It is secondary to placental transfer of maternal alloantibodies directed against paternally inherited antigens present on the fetal platelets, but absent on maternal platelets. When this type of incompatibility between parental platelet exists, the mother may develop an immune response to antigens expressed on the fetal platelets inherited from the father. Homozygosity of the father for a specific platelet antigen results in the expression of that antigen on the fetal platelets; however, heterozygosity of the father for that antigen results in approximately 50% incidence of neonatal alloimmune thrombocytopenia [20].

Antigens appear on fetal platelets early in the gestation and maternal antibodies are able to cross the placenta early in the second trimester, thereby reacting with antigens on fetal platelets and inducing severe fetal thrombocytopenia. Mothers do not generally have a history of abnormal bleeding and have a normal platelet count. Neonatal alloimmune thrombocytopenia is usually a consequence of antibodies against the antigen HPA-1a, but there are also other antigens [21].

The severity of thrombocytopenia is unpredictable. Nevertheless certain clinical features are characteristic of neonatal alloimmune thrombocytopenia, such as severe thrombocytopenia (20,000/μL–50,000/μL) at birth with a platelet count that continues to fall over the first days of life and then increases over the next 1–4 weeks. Intracranial hemorrhage occurs in approximately 10–15% of cases; at least one half of these occur in utero, with death occurring in 10% of affected infants and neurological sequelae in 20%. Although controversial, prenatal treatment with percutaneous umbilical cord blood sampling and administration of IVIG to the mother may influence the natural history of the disease. Neonatal alloimmune thrombocytopenia causes more intracranial hemorrhage than any other hemostatic disorder in the newborn period. The usual presentation of an infant born to healthy mother is with purpura and/or petechiae within a few hours of birth, and severe thrombocytopenia without other signs of disseminated intravascular coagulopathy (DIC) or sepsis. The full blood count is usually normal in the affected neonates apart from the severe thrombocytopenia [20].

In addition to a low platelet count, usually due to platelet consumption and rarely associated with reduced production, an infant's platelets may also have defective function [22]. The treatment of choice for rapidly raising the platelet count in neonates with severe neonatal alloimmune thrombocytopenia is transfusion with maternal or HPA-matched platelets because random donor platelet transfusions usually do not improve, or only briefly improve, the platelet count. Maternal platelets are usually washed to remove plasma, which may contain antiplatelet antibodies. IVIG can be used in the neonate to augment the platelet count, even though first improvement in platelet count may not be evident for 24–72 hours [20].

Autoimmune thrombocytopenia occurs in neonates born to mothers with idiopathic thrombocytopenic purpura (ITP), systemic lupus erythematosus (SLE), lymphoproliferative disorders, or hyperthyroidism. Maternal autoantibodies bind to the maternal and the fetal platelets and result in platelet destruction [22]. Thrombocytopenia occurs in approximately 10% of cases in which the mother has ITP or SLE, and intracranial hemorrhage occurs in approximately 1% of these. The mother is usually healthy without a history of ITP or other autoimmune disorder, but with a slightly decreased platelet count that resolves after delivery. Autoimmune thrombocytopenic purpura in pregnant women can induce moderate or severe thrombocytopenia in the fetus or the newborn, but the severity of the maternal disease and maternal platelet count usually predicts the neonatal platelet count [23].

The neonate born to a mother with autoimmune disease should have platelet counts monitored for at least the first week. The usual clinical picture includes mild to moderate thrombocytopenia, petechiae or bruising in an otherwise healthy neonate. Intracranial hemorrhage is very rare. Thrombocytopenia usually persists 1–2 months in the infant, with spontaneous resolution.

The severity of thrombocytopenia in the first infant delivered to a woman with active ITP usually predicts the degree of thrombocytopenia of the next sibling. Monitoring the course of thrombocytopenia in breastfed neonates of mothers with ITP is recommended, since IgG antibodies are transferred via breast milk, and Bussel has reported one neonate in whom severe neonatal thrombocytopenia resolved only after stopping breastfeeding [20, 24].

Treatment of infants affected by maternal ITP may include intravenous immunoglobulin (IVIG) or steroids, or a combination of IVIG and steroids, platelet transfusion, or exchange transfusion [1].

Infections

Thrombocytopenia frequently accompanies systemic neonatal infection, even in the absence of disseminated intravascular coagulation (DIC). The reported incidence of thrombocytopenia associated with sepsis in neonates is extremely variable,

depending on the definition of thrombocytopenia; 80% of neonates if thrombocytopenia is diagnosed as a platelet count of less than 150,000/μL, 55–65% if thrombocytopenia is diagnosed as a platelet count of less than 100,000/μL.

The usual mechanism responsible for thrombocytopenia in infected neonates is accelerated platelet destruction, which may be caused directly by the organism or its products, immune-mediated destruction, and adhesion of the platelets to the subendothelial layer of injured blood vessels [25].

Twenty-five percent of neonates with a bacterial infection are thrombocytopenic at the time sepsis is diagnosed. Thrombocytopenia may develop after 36–48 hours but bleeding is rare because this variety of thrombocytopenia is usually moderate. However, monitoring the platelet count is recommended, since these patients may require platelet transfusions. Recovery occurs over 5–7 days as the sepsis improves [26].

Fungal sepsis, which is a particularly common problem among extremely-low-birth-weight neonates is frequently associated with thrombocytopenia. In contrast to the thrombocytopenia that may complicate bacterial infection, it is generally severe with platelet counts falling below 50,000/μL. Antifungal treatment is recommended while awaiting culture results in a clinically septic extremely low-birth-weight infant with thrombocytopenia [27].

Thrombocytopenia is common with congenital viral infections. All the viruses that cause TORCH infections, coxsackie virus B, Epstein Barr virus, adenovirus, and echovirus can cause thrombocytopenia. The mechanism is most likely a combination of accelerated destruction, diminished production and accelerated removal of platelets from the circulation because of the reticuloendothelial hyperactivity and splenomegaly that often accompany congenital viral infections. Vacuolization of megakaryocytes has also been reported [28].

Disseminated Intravascular Coagulation

Disseminated intravascular coagulation (DIC) is a disorder of hemostasis, with an inappropriate systemic activation of normal clotting mechanisms after endothelial injury, which is secondary to the underlying disease, which may include infection, shock, anoxia, renal vein thrombosis, necrotising enterocolitis and meconium aspiration. All these disease processes are complicated by endothelial disruption and result in release of tissue factors and cytokines, and excessive activation of coagulation and fibrinolysis, and, ultimately, a consumptive coagulopathy [29].

Infants with DIC have low platelet counts and fibrinogen levels, prolonged prothrombin time (PT) and partial thromboplastin time (PTT), and elevated fibrin degradation products. They often present with gastrointestinal bleeding, bleeding from venipuncture sites, or pulmonary hemorrhage. Successful treatment of neonatal DIC depends on a correct diagnosis, treatment of the underlying disorders, and maintaining platelet counts greater than 50,000/μL, fibrinogen concentrations greater than 1 g/L and PT values within the physiological range.

Necrotizing Enterocolitis

Thrombocytopenia is common in neonates with necrotising enterocolitis (NEC). Platelet counts frequently fall to the range of 30,000–60.000/μL, and platelet transfusions do not usually maintain a normal blood platelet concentration for more than 1 or 2 days, suggesting that platelet destruction is the chief mechanism [1].

Asphyxia

Placental insufficiency can be associated with early-onset thrombocytopenia in preterm neonates, and perinatal hypoxia has been associated with severe and prolonged thrombocytopenia. Some of these infants have a decreased platelet count without DIC or persisting beyond the resolution of DIC. The decreased platelet count is probably due to the direct harmful effects of hypoxia on the megakaryocyte progenitors [30].

Thrombosis

Thrombocytopenia sometimes occurs in neonates who have a localized thrombus in the renal vein, or attached to an indwelling catheter, or in the ECMO circuit. It is unclear whether the thrombocytopenia is caused by platelet adhesion and aggregation to a developing thrombus, or by platelet consumption at the vascular surfaces where the thrombus has begun.

Some sick neonates develop thrombocytopenia without any other coagulation defect, associated with platelet thrombi on the cardiac valves. In some neonates, platelets which have aggregated in the pulmonary capillaries release substances causing vasoconstriction with consequent pulmonary hypertension [29].

Kasabach-Merritt Syndrome

Consumption of platelets in giant hemangiomata can cause thrombocytopenia. These vascular tumors are usually solitary and may involve internal organs, head or neck, the trunk, or the extremities. Platelets are trapped by abnormal endothelium within the hemangioma, and the platelet counts often fall to less than 50,000/μL. In some patients there is laboratory evidence of DIC but thrombocytopenia may also occur in the absence of DIC. Proposed treatments have included steroids, alpha interferon, total or partial surgical excision of the hemangioma, or embolisation or irradiation of the hemangioma [31].

Drugs Producing Thrombocytopenia in Mother and Fetus

Thrombocytopenia as a result of maternal-fetal drug interaction is thought to be caused by immune reactions in which antibody is formed against a drug-haptene complex that cross-reacts with a platelet antigen. This has been reported with the maternal use of quinine, carbamazepine, phenytoin and valproic acid [14]. Thrombocytopenia induced by the maternal use of thiazide diuretics has also been reported and may be due to impaired fetal platelet production with reduced megakaryocytes in the marrow but without inducing thrombocytopenia in the mother. *In vitro* studies have described thrombocytopenia with the maternal use of ampicillin and furosemide [1].

102.5 Qualitative Disorders of Platelets

Qualitative disorders of platelets form a large group of rare diseases in which the infant has a normal platelet count but excessive mucocutaneous bleeding. The qualitative platelet disorders are classified as either hereditary or acquired.

102.5.1 Hereditary Disorders

Most hereditary disorders of platelet function do not cause problems during the neonatal period but present later in life. Glanzmann's thrombasthenia is one type of hereditary platelet dysfunction that may present during the neonatal period. A deficiency of the glycoprotein IIb/IIIa complex, which is inherited in an autosomal recessive pattern, results in platelets that do not aggregate in response to normal stimuli, except to ristocetin. The microscopic appearance of the platelets and the platelet count are normal [32]. Although platelet transfusions are effective, they should be used only for serious hemorrhage or if there is a need for surgery because these patients often develop alloantibodies. Recombinant factor VIIa may provide a therapeutic alternative to platelet transfusion for some patients [33].

Bernard-Soulier syndrome is an autosomal recessive disorder resulting from a deficiency of platelet glycoprotein protein Ib, which mediates the initial interaction of platelets with subendothelial components via the von Willebrand protein. This syndrome can result in a severe bleeding disorder. Platelets do not aggregate in response to ristocetin. The platelet count is low, but the platelets are usually large, often the size of red blood cells. As in Glanzmann's thrombasthenia, platelet transfusions may cause antibody formation to the absent glycoprotein, and the administration of recombinant factor VIIa for acute bleeding episodes should be considered as an alternative treatment.

May-Hegglin anomaly is a benign condition characterised by giant platelets. Giant platelets are also found in variants of Alport's syndrome. Platelet function disorders relating to platelet secretion cause mild to moderate bleeding. Included in this subset of disorders are Gray Platelet syndrome, Dense Granule deficiency, Chediak-Higashi syndrome, and Hermansky-Pudlak syndrome [1].

102.5.2 Acquired Disorders

Acquired disorders of platelet function are common. A primary hemostatic defect can occur as a result of medications given to the mother or neonate. Aspirin, given to the mother, crosses the placenta and may produce irreversible inhibition of the cyclooxygenase enzyme, increasing the bleeding time for the life of affected platelets. Gastrointestinal bleeding, melena, or cephalohematoma may affect neonates whose mothers received aspirin during pregnancy within 5 days of delivery [1].

Indomethacin and ibuprofen, commonly used for the closure of patent ductus arteriosus, can interfere with cyclooxygenase function. The influence of these drugs on platelet function is transitory, generally lasting about 24 hours. Ibuprofen, administered to neonates with a PDA, has little adverse effect on platelet plug formation, and prolongs the bleeding time less than indomethacin [34, 35]. Nitric oxide (NO) also has the potential to reduce platelet function, probably because of increased cGMP. In premature infants, inhaled NO may prolong bleeding time, decrease platelet aggregation, and inhibit platelet adhesion to endothelial cells, increasing the risk of intraventricular hemorrhage [36].

Extracorporeal membrane oxygenation (ECMO) is used in neonates for the treatment of persistent pulmonary hypertension, meconium aspiration, and congenital diaphragmatic hernia. Impaired platelet function and thrombocytopenia have been described in patients on ECMO. During ECMO, platelet dysfunction persists despite platelet transfusions, and platelet function returns to normal 8 hours after thediscontinuation of ECMO [1].

Other circumstances where platelet dysfunction has been described include hyperbilirubinemia and phototherapy, although bleeding has not been reported in otherwise healthy infants in these situations [1].

Uremia and/or renal failure can also cause neonatal platelet dysfunction. Gastrointestinal tract bleeding is the most frequent symptom, and bleeding time is often very prolonged in these patients. Excessive production of nitric oxide by endothelial cells inhibits platelet function, and may be responsible for bleeding in uremic patients. Because the prolonged bleeding time and the hemostatic abnormalities may be partly corrected by red blood cell transfusion or erythropoietin therapy, the failure of hemoglobin to turn off excess

nitric oxide synthesis has been suggested as a cause of the platelet dysfunction. Another suggestion is that platelets arrive on exposed vascular subendothelium, being propelled there mainly by erythrocytes, which release ADP to activate the platelets following vessel injury. Consequently, a low hematocrit may result in a prolonged bleeding time [37].

102.6 Evaluating Platelet Function in a Neonate

The main goal of platelet function testing is to determine the cause of abnormal bleeding. However, a thorough assessment of platelet function in the neonate is difficult because of difficulties in obtaining sufficient blood for these types of studies [16]. This is particularly true for aggregometry and flow cytometric evaluation of platelet activation, but various other studies, such as those listed below, can be helpful.

102.6.1 Laboratory Studies

102.6.1.1 Peripheral Smear

Examination of the peripheral smear is useful for evaluating platelet clumping or platelets adhering to neutrophils (platelet satellitism), or Bernard-Soulier syndrome with giant platelets.

102.6.1.2 Platelet-Associated Immunoglobulin G

Assays have been developed to assess the presence of IgG on platelets. In the thrombocytopenic neonate this may indicate platelet destruction and reduced platelet survival. The detection of increased platelet-associated immunoglobulin is observed in a variety of conditions associated with thrombocytopenia, which limits the value of this test for the diagnosis of specific disorders, such as ITP.

102.6.1.3 Bleeding Time

The bleeding time measures the time required for a platelet plug, which occurs through the interaction of platelets with the subendothelial structures of a damaged vessel, to arrest bleeding from a standardised superficial skin incision [38, 39].

102.6.1.4 Platelet Function Analyzer 100

The platelet function analyzer 100 (PFA-100) is an *in vitro* method for the assessment of primary hemostasis under shear stress, where primary hemostasis is simulated by an *in vitro* quantitative measurement of platelet adhesion, activation and aggregation in whole blood. The PFA-100 uses a disposable test cartridge that contains a membrane coated with collagen and either ADP (Col/ADP membrane) or epinephrine (Col/Epi membrane), thus mimicking exposed subendothelium. A blood sample of 0.8 mL of citrated blood is placed in a cup and is aspirated through the aperture. The shear stress and the agonists in the membrane activate platelets lead to platelet aggregation. The end point, expressed as closure time, is when blood flow stops because of occlusion of the aperture by platelet aggregates, giving a measure of platelet dependent hemostasis [40]. The closure times, similar to bleeding times, are significantly shorter in neonates than in adults and healthy children. The advantages of this instrument include simplicity and reproducibility.

102.6.1.5 Mean Platelet Volume

The mean platelet volume (MPV) is a measure of the average size of circulating platelets. Since the average platelet size is larger when the marrow produces increased numbers of platelets, high MPV in thrombocytopenic neonates can be an indirect measure of increased platelet production in bone marrow.

102.6.1.6 Reticulated Platelet Count

Reticulated platelets, similar to reticulated erythrocytes, lose their RNA content in the process of aging and senescence. Because reticulated platelets are detectable in the circulation for only 24 hours, they are an excellent measure of platelet production and turnover. These new platelets can be recognized by flow cytometry, and in term neonates they represent 3–5% of circulating platelets. Increased production of platelets is expected to increase the percentage of reticulated platelet [41].

102.6.1.7 Bone Marrow Biopsy

In most cases of platelet disorders, bone marrow biopsy is not necessary. It provides important information about the state of platelet production that is not otherwise available. It is particularly useful in evaluating neonates with thrombocytopenia when an estimate of the marrow megakaryocyte mass is needed. Unlike a direct aspiration of cells from a neonate's bone marrow, which can result in a sample that is so diluted with peripheral blood that interpretation is limited, the marrow biopsy preserves the basic architecture of the marrow. Bone marrow biopsy uses a 19 gauge, half-inch Osgood needle to obtain bone marrow clots from the tibia of a baby, enabling the assessment of marrow cellularity and architecture [42].

102.7 Treatments

102.7.1 Platelet Transfusion

Platelet transfusions represent the only specific therapy currently available for most thrombocytopenic neonates. There is great variability worldwide in neonatal platelet transfusion practice, due to the lack of concrete evidence to guide transfusion decisions [43–46]. In approximately 75% of neonates with thrombocytopenia, the condition is mild and transient and needs no treatment. However, in 20–25% of cases, one or more platelet transfusions are given in an attempt to treat or decrease the risk of hemorrhage. Among critically ill patients, most platelet transfusions are given to prevent, rather than to treat, bleeding, with a transfusion trigger of 40,000 – 50,000/μL. It is not clear how many platelet transfusions in the NICU are actually unnecessary, or provide more risk than benefit [43, 47].

Baer and coworkers reported that the number of platelet transfusions administered in the NICU predicts the mortality rate, suggesting that multiple platelet transfusions may be harmful. Since platelet transfusions carry risks, eliminating unnecessary platelet transfusions would be useful [48]. Improving transfusion practice requires the definition of a safe lower limit for platelet counts in stable neonates, platelet transfusion strategies for critically ill neonates, and improved treatments for conditions that are associated with severe thrombocytopenia. To avoid unnecessary transfusions, Christensen et al proposed transfusion guidelines based on platelet mass (platelet count times mean platelet volume). In a pilot study, the use of these guidelines was associated with fewer platelet transfusions without any increase in problems due to hemorrhage [49, 50].

While there is no scientific evidence to guide platelet transfusion practices in neonates, individual NICUs should adopt guidelines appropriate to their practice, and adhere to them closely [50]. As a general rule, platelets should be given to non-bleeding thrombocytopenic neonates as prophylactic transfusions to maintain platelet count > 20,000/μL for clinically stable neonates, and > 50,000/μL for clinically unstable neonates. Platelet counts should be maintained > 50,000/μL to 100,000/μL when there is significant bleeding and > 50,000/μL for invasive procedures [51].

102.7.2 Thrombopoietic Factors

Inadequate megakaryocyopoiesis and platelet production are major contributors to most episodes of neonatal thrombocytopenia. As alternatives to platelet transfusion, the administration of thrombopoietic growth factors, such as recombinant thrombopoietin (rTPO) and interleukin 11 (IL-11) may be useful. rTpo and IL-11 are promising agents for older patients, but both have potential toxicities that are likely to preclude their use for sick preterm infants.

102.7.3 Other Therapies

Recombinant activated factor VII has been used successfully off-label in controlling life-threatening bleeding during infancy, but the clinical situations in which benefits are likely, and potential toxicities, particularly thrombosis, remain unknown. The administration of intravenous immunoglobulin (IVIG) is useful for the treatment of immune thrombocytopenia. Corticosteroids were classically used for alloimmune and autoimmune neonatal thrombocytopenia, often in combination with IVIG, but are now unusual. Dosing schedules for IVIG include 400 mg/kg/d for 3–5 consecutive days or a single dose of 1000 mg/kg on day 1.

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Anemia in the Neonatal Period

Robert D. Christensen and Robin K. Ohls

103.1 Introduction

The term anemia refers to a pathological reduction in the hematocrit, blood hemoglobin concentration, and circulating erythrocyte count [1, 2]. These three laboratory measures are somewhat similar to one another, in that each quantifies the same biological variable involving the capacity of blood to deliver oxygen to tissues. However, none of the three measurements actually assesses whether oxygen demands of tissues are being adequately met. In fact, limitations in delivery of oxygen to a neonate's tissues are frequently not the result of anemia at all, but instead are due to abnormalities in oxygen intake from pulmonary pathology. This chapter reviews the various pathologies that give rise to anemia during the neonatal period, and provides practical approaches for dealing with these clinical issues.

103.2 Erythropoietin – Biological Considerations in the Fetus and Neonate

During early fetal and neonatal development, erythropoietin is more than an erythropoietic growth factor. Erythropoietin (Epo) is a constituent of amniotic fluid in concentrations of 25–40 mU/mL. A normal human fetus swallows 200–300 mL of amniotic fluid/kg/day and thus swallows 10–15 U of Epo/kg/day [3, 4]. In humans, Epo does not cross the placenta, and the source of the Epo in amniotic fluid is not the maternal circulation. In the second and third trimesters, amniotic fluid is largely derived from fetal urine, with minor constituents from fetal tracheal effluent and the placenta and fetal membranes. However, Epo in amniotic fluid does not come from fetal urine. The fetal kidney makes little Epo before delivery, and the first-voided urine of neonates generally has no detectable Epo [3]. Studies using in situ hybridization and immunohistochemistry indicate that the source of Epo in amniotic fluid is largely maternal: from mesenchymal and endothelial cells in the deciduae and from the amnion [4].

Epo is also present in colostrum and breast milk in concentrations of 10–20 mU/mL [3, 5, 6]. Epo levels in milk do not correlate with Epo levels in the mother's blood [5, 6]. In fact, over the first weeks of lactation, mother's serum Epo concentrations fall, while her milk Epo concentrations increase, reaching the highest concentrations in women breastfeeding for a year or more. The source of Epo in breast milk appears to be mammary gland epithelium [4–6].

Epo in human amniotic fluid, colostrum, and breast milk is relatively protected from proteolytic digestion in the fetal and neonatal gastrointestinal tract. Rather than being absorbed from the gastrointestinal track into the blood, the Epo swallowed by the fetus and neonate binds to Epo receptors on the luminal surface of villous enterocytes, where it serves as a growth and development factor. Experimental animals artificially fed formulas devoid of Epo have retarded villous development, a condition that can be remedied by enteral recombinant Epo and blocked by antiEpo antibody [4].

Epo is produced by cells in the developing central nervous system and is present in relatively high concentrations in fetal cerebrospinal fluid (CSF) [7–12]. The highest concentrations of Epo in the CSF are in the most premature neonates, and by several years of age CSF Epo concentrations are generally below 1 mU/mL [12]. Epo receptors are expressed on human fetal neurons [13–15], and at least small quantities of recombinant Epo administered intravenously cross the blood brain barrier and appear in the CSF [16]. Epo is a neuroprotectant [8–10]. Its production increases rapidly in the brain during hypoxia, and when Epo binds to receptors on neurons, antiapoptotic activity is induced. The clinical utility of recombinant Epo as a neuroprotectant is a topic of current studies.

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

The liver is the primary site of fetal Epo production. The kidney produces only about 5% of the total Epo during midgestation. The mechanisms regulating the switch in Epo production from the liver to the kidney are not completely known but may involve developmental expression of transcription activators such as hypoxia inducible factor and hepatic nuclear factor 4 [18, 19], or developmental methylation of promoter and enhancer regions. Alternatively, the switch might involve the GATA transcription factors, particularly GATA-2 and GATA-3, which are negative regulators of Epo gene transcription.

103.3 Normal Erythrocyte Values During Human Fetal Development

"Reference ranges" are generally used in Neonatology in place of "normal ranges" commonly used in adult medicine. The difference is, reference ranges are constructed from clinically-obtained laboratory tests, not from tests performed on healthy volunteers. In order to more closely approximate a normal range, the laboratory tests included in a reference range include only patients with minimal pathology or with pathology not known to be related to the test under consideration. For instance, reference ranges for the hematocrit of neonates excludes data from neonates with clinical issues known to affect the hematocrit, such as PRBC transfusion recipients, those with hemolytic disease, or those with a reduction transfusion. Reference ranges for hematocrit and hemoglobin on the day of birth [1] are shown in Fig. 103.1.

Circulating erythrocytes in the fetus have features reminiscent of "stress erythropoiesis" in adults. These features include marked anisocytosis, pokilocytosis, macrocytosis, and the presence of nucleated erythrocytes. Marrow cellularity in the fetus is relatively high. Erythroid precursors account for 30–65% and myeloid cells 45–75% of nucleated marrow cells at birth [20]. The myeloid to erythroid ratio at birth is approximately 1.5:1. Marrow cellularity decreases after birth, attaining a density that is normal for adults by 1–3 months. Initially, this decrease in cellularity results from a rapid decline in red cell production. At 1 week of age, erythroid elements account for only 8–12% of nucleated cells, and the myeloid to erythroid ratio exceeds 6:1. The normal adult proportion of myeloid to erythroid precursors is not established until the

Table 103.1 Characteristics of neonatal erythrocytes

A. Characteristics explained by large and young erythrocytes
   1. Increased levels of ATP
   2. Increased consumption of glucose and galactose
   3. Increased enzymatic activity
      a. Aldolase
      b. Galactokinase
      c. Galactose-1-phosphate uridyl transferase
      d. Glucose-6-phosphate dehydrogenase
      e. Glutathione reductase
      f. Glyoxalase I and II
      g. Hexokinase
      h. Lactic dehydrogenase
      i. Phosphoglycerate mutase
      j. Pyruvate kinase
      k. Triosephosphate isomerase

B. Characteristics not explained by large cell size, but unique to neonatal erythrocytes
   1. Embden-Meyerhof pathway
   2. Pentose-phosphate pathway and glutathione metabolism
   3. Nonglycolytic enzymes
      a. Adenylate kinase
      b. Carbonic anhydrase
      c. Catalase
      d. Cholinesterase
      e. Cytochrome b5 reductase
      f. Decreased enzyme activity
      g. Phosphoribosyl transferase

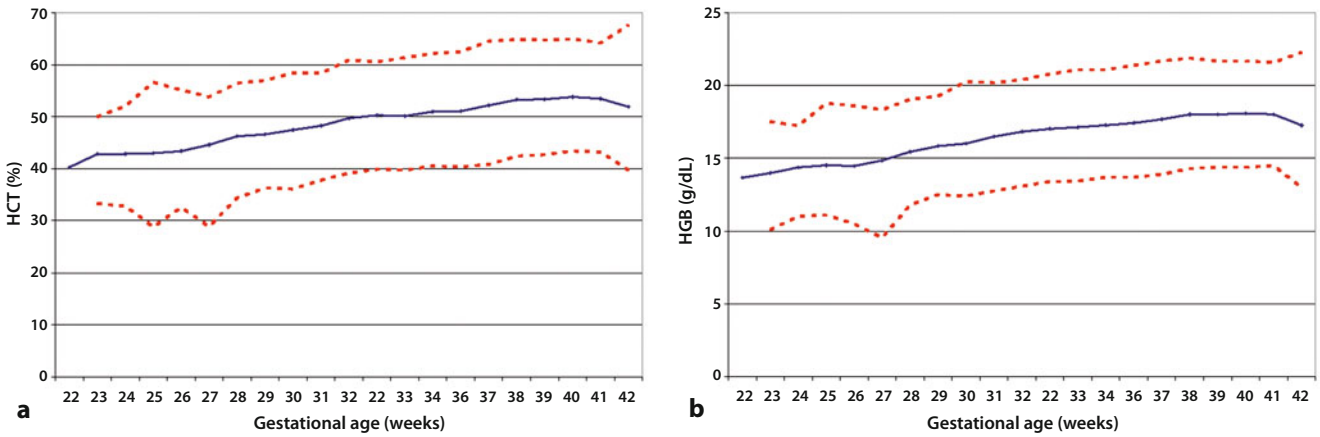

Fig. 103.1 Reference ranges for hematocrit (**a**) and blood hemoglobin concentration (**b**) on the day of birth. Values are given as a function of gestational age. HCT Hematocrit, HGB Hemoglobin. Reproduced from [1], with permission

third month. Both the percentage and absolute number of lymphocytes increase during the first 2 months, so that by 3 months of age, they constitute nearly 50% of nucleated marrow cells. Differential counts of bone marrow aspirates from preterm infants are similar to those of term infants [21].

In newborn infants, the hemoglobin (Hb) concentration and hematocrit of capillary blood are 5–10% higher than those of venous blood [1]. The difference between capillary and venous values is greatest at birth but disappears by 3 months of age. The discrepancy is greatest in preterm infants and in those with hypotension, hypovolemia, and acidosis [22–24].

Reticulocytes at birth are approximately 5% of erythrocytes, with a range of 4–7% [22, 25]. Reticulocytes remain elevated for the first 1–3 days, dropping abruptly to 0–1% by day 7. Nucleated red blood cells (NRBC) are seen regularly on blood smears during the first day of life constituting about 0.1% of the red cell population (500 normoblasts/mm3) [22]. The absolute count of NRBC decreases with advancing gestational age. It has been observed that the mean value of NRBC varies from 5643.3 (SD 7228.2) in newborns of 26.6 weeks of gestation to 441.6 (SD 807.3) in newborns of 38.6 weeks [26]. The finding of higher than normal content of NRBC in cord blood, in relation to the gestational age, strongly suggests that the fetus had severe hypoxia several hours before the birth. The increased NRBC production by the hypoxic fetus reflects a higher erythropoietin production as an answer to low tissue oxygen availability [27]. NRBC are not common in the circulation after the first 3 days unless intermittent or chronic hypoxemia is present.

Red cell morphology is characterized by macrocytosis and poikilocytosis. Target cells and stomatocytes are particularly prominent. Similarly a high proportion of siderocytes (3.2% versus normal adult mean of 0.1%) are seen [28, 29].

Measuring the circulating red blood cell volume in a fetus or neonate is difficult. Mock et al used a nonradioactive method, based on *in vivo* dilution of biotinylated RBC enumerated by flow cytometry, to estimate the correlation between hematocrit and circulating RBC volume in infants below 1300 grams and found that venous hematocrit values correlated highly with the circulating erythrocyte volume ($r = 0.907$; $p < 0.0001$) [30, 31].

Neonates have a shorter red cell survival than do children and adults. The life span of red cells from term infants is estimated to be 60–80 days, using the 51Cr method [32] and 45–70 days using methods involving 59Fe [33]. Fetal studies using [14C] cyanate-labeled red cells in sheep revealed an average red cell life span of 64 ± 6 days [34]. The mean red cell life span increased linearly from 35–107 days as the fetal age increased from 97 days (midgestation) to 136 days (term).

Neonatal red cells transfused into adults have a short survival [33], indicating that factors intrinsic to the newborn red cell are responsible. This conclusion of factors intrinsic to the newborn red cells gains further support by the demonstration that adult red cells survive normally in newborn recipients [34]. The life span frequency function is not parametrically distributed, in that most cells are destroyed before the mean survival is reached. Shortened red cell survival corresponds with erythropoietic rates at birth that are three to five times greater than those of normal adults.

The abrupt transition from the relative hypoxia of the uterus to an oxygen-rich environment triggers responses that have profound effects on erythropoiesis (Fig. 103.2). During the first 2 months of life, the infant experiences both the highest and lowest Hb concentrations occurring at any time in development. Epo levels at birth are usually well above the normal adult range and fall in the immediate postnatal period [35]. By 24 hours, the Epo value is below the normal adult range where it remains throughout the first month of life. The decrease in Epo is followed by a decline in the number of bone marrow precursors [36] and a fall in the reticulocyte count.

The combination of shortened cell survival, decreased production, and growth related expansion of the blood volume is responsible for a progressive fall of the Hb concentration to a mean of approximately 11 g/dL at 2 months of age [1]. The lower range of normal for infants of this age is approximately 9 g/dL. This nadir is called physiologic anemia, in that it is not associated with apparent distress and is not prevented with nutritional supplements. Stabilization of the Hb concentration is heralded by an increase in reticulocytes at 4–8 weeks. Thereafter, the Hb concentration rises to a mean level of 12.5 g/dL, where it remains throughout infancy and early childhood [21, 22].

At term, the placenta and umbilical cord contain 75–125 mL of blood (30–40 mL/kg), or approximately 1/4–1/3 of the fetal blood volume. Umbilical arteries constrict shortly after birth, but the umbilical vein remains dilated, and blood flows in the direction of gravity. Infants held below the level of the placenta can receive half of the placental blood volume (30–50 mL, or 10–15 mL/kg) in 1 minute. Conversely, infants held above the placenta can lose 20–30 mL of blood back into the placenta per minute [37, 38]. The blood volume of infants with early cord clamping averages 72 mL/kg, whereas the volume of infants with delayed cord clamping averages 93 mL/kg.

Linderkamp et al compared postnatal alterations in blood viscosity, hematocrit, plasma viscosity, red cell aggregation, and red cell deformability in the first 5 days of postnatal life in full-term neonates with early (less than 10 seconds) and late (3 minutes) cord clamping [37, 38]. The residual placental blood volume decreased from 52 ± 8 mL/kg of neonatal body weight after early cord clamping to 15 ± 4 mL/kg after clamping. The neonatal blood volume was 50% higher in the late cord-clamped infants. Intrauterine asphyxia appears to have little effect on blood volume, whereas intrapartum asphyxia and nuchal cords are associated with a reduced blood volume [39, 40].

Placental transfer of blood to preterm infants occurs by delayed clamping of the umbilical cord. Transfer of about 10 mL/kg body weight can be expected by delaying clamping for 30–60 seconds and has been claimed to reduce intraventricular hemorrhage and late-onset sepsis [39–41]. However,

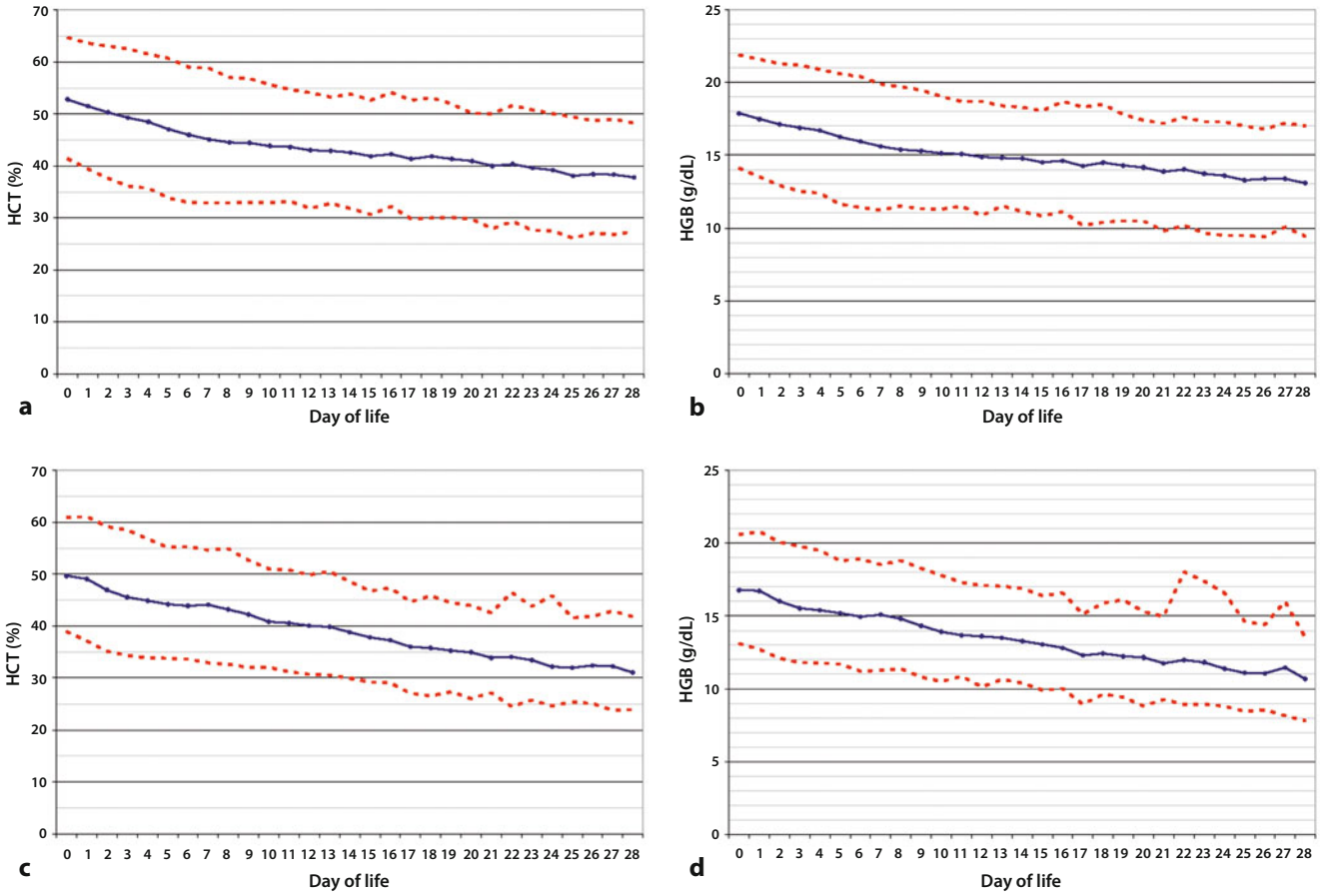

Fig. 103.2 Reference ranges for hematocrit and blood hemoglobin concentration during the first month after birth. **a** Hematocrit from late preterm and term neonates. **b** Hemoglobin from late preterm and term neonates. **c** Hematocrit from preterm neonates. **d** Hemoglobin from preterm infants. *HCT* Hematocrit, *HGB* Hemoglobin. Reproduced from [1], with permission

in actual practice, preterm deliveries often have rapid cord clamping in order the pass the neonate to the neonatal team for resuscitation.

103.4 Fetal and Neonatal Anemia Due to Abnormalities in the Erythrocyte Membrane or Cellular Metabolism

Neonates have erythrocyte membranes that differ slightly from those of adults [42]. The percentage of spectrin dimers is the same as in adult cells but neonatal cells have more immunoreactive myosin [43]. The quantity and distribution of lipids in neonatal red cells differ in several respects from adult red cells. Total lipids, phospholipid, and cholesterol are increased out of proportion to the surface area of newborn red cells [44].

Antigen expression in neonatal cells differs from that of adult cells. The A, B, S, and Lutheran antigens are present in lower amounts, membrane receptors for digoxin are 2.5 times that in adult red cells, and an increase in insulin receptors is also seen, possibly explainable by their larger surface area [45]. Red cell deformability and viscoelastic properties are normal [57], but the passage of newborn red cells through small pore filters is impaired. This is probably the result of larger size. Osmotic fragility of erythrocytes from preterm neonates is similar to or less than erythrocytes of term neonates. However, preterm neonates may have a small subpopulation of cells more susceptible to hemolysis, giving them a greater tendency to develop hemolytic jaundice [45, 46].

Red cells of neonates have distinctive metabolic characteristics [47–49]. To some extent these differences are explained by the young age and increased size of the red cells. The increase in activity of many of the glycolytic enzymes is comparable in magnitude to that observed in high-reticulocyte adult blood. Increased glycolytic enzyme activity is responsible for increased consumption of glucose and galactose and increased levels of adenosine triphosphate (ATP).

The concentration of 2,3-DPG falls rapidly during short periods of incubation [49], apparently because of accelerated breakdown. Preterm infants have lower 2,3-DPG concentrations than term infants. These concentrations gradually increase with gestation. Concentrations can be increased with the use of Epo, thereby shifting the oxygen dissociation curve to the right [50].

The activities of two key enzymes of the pentose phosphate pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, are increased due to the young mean red cell age. The response of the pentose phosphate pathway to oxidant stimuli is normal, and the level of reduced glutathione is equal to or greater than that found in adults [51]. However newborn cells have glutathione instability, increased Heinz body formation, and a propensity to increased methemoglobin generation, all indicative of greater susceptibility to oxidant-induced injury. This characteristic, which has been known for a long time [52], has been better explained by recent investigations. It has been demonstrated that red cells of newborns and particularly of premature infants have high intraerythrocyte release of non-protein bound iron and evident binding of autologous IgG to band 3 dimers [53]. These markers of oxidative stress demonstrated that red cell injury is enhanced by hypoxia and acidosis [54]. Therefore, the demonstration of increased oxidative cell injury of red cells during hypoxia strongly suggests a role of hypoxia induced cell damage in the reduced life span of the fetal and neonatal erythrocytes. The high susceptibility of neonatal erythrocytes to oxidative stress does not appear to only depend upon low levels of glutathione peroxidase and glutathione synthetase, no apparent relationships have been clearly demonstrated to exist between these deficiencies and clinical evidence of red cell vulnerability.

Although elevated at birth, the red cell ATP level falls rapidly during short periods of incubation. The uptake of labeled orthophosphate by cord blood cells is slower than that by adult cells, resulting in delayed incorporation of phosphate into ATP and 2,3-DPG [50, 55].

Cord blood contains three types of Hb: HbF (α_2, γ_2), HbA (α_2, β_2), and HbA2 (α_2, δ_2). HbF constitutes the major fraction (50–85%). Because of this, hemoglobinopathies involving β-chain synthesis, such as sickle cell disease and β-thalassemia, are not problematic during the neonatal period. The G-γ to A-γ ratio at birth is approximately 3:1, in contrast to a ratio of 2:3 in adults [55, 56]. HbA accounts for 15–40% of the Hb at birth. HbA2 is only present in trace amounts (mean, 0.3%) at birth, but it increases slowly after birth, reaching the normal adult level (2–3%) by 5 months of age. Relative to values in adults, free erythrocyte protoporphyrin is high at birth and remains elevated through the first six months.

The level of HbF at birth is influenced by a number of variables, the most significant of which is gestational age. Premature infants have more HbF and postmature infants less [55]. Neonates who have survived chronic intrauterine hypoxia have higher levels of HbF. Hemolytic disease is associated with lower levels of HbF. This association does not result from a difference in the synthetic ratios of β- and δ-globin chains, but rather relates to a younger population of red cells produced late in fetal life. The switch from γ-chain synthesis to β-chain synthesis is insensitive to environmental variables and appears to be developmentally programmed. Neither intrauterine transfusion nor neonatal exchange transfusion affects the synthetic rates of β- and δ-chains. Studies quantifying the specific globin mRNAs report that (G)gamma globin mRNA to total gamma globin mRNAs remains around 66% until the 44th week of post-conceptional age, when a change in the (G)gamma and (A)gamma globin mRNA proportions occurrs. Immature red cells of adults have a range of (G)gamma globin mRNA to total gamma globin mRNAs varying from 20–74%.

HbF has a higher affinity for oxygen than does HbA [55]. The oxygen tension at which the Hb of cord blood is 50% saturated is 19–21 mmHg, 6–8 mmHg lower than that of normal adult blood. This shift to the left of the Hb-oxygen dissociation curve results from poor binding of 2,3-DPG by HbF. The position of the oxygen dissociation curve is determined by both the percentage of HbA and the red cell content of 2,3-DPG. As the relative proportion of HbA increases, the oxygen dissociation curve shifts by approximately 4–6 months of age to the adult position. The increased oxygen affinity of HbF confers a physiologic advantage to the fetus in facilitating the transfer of oxygen from mother to fetus.

HbF is resistant to acid elution. This property forms the basis for its chemical quantitation. Unlike HbA, HbF is not eluted from fixed blood smears immersed in an acid buffer [55]. This property permits the differential staining of HbF and HbA, a technique widely used to study the distribution of HbF in red cells and to detect fetal cells in the maternal circulation, termed a Kleihauer Behke stain.

When a neonate has Coombs negative hemolytic jaundice, considerations include an inherited disorder of erythrocyte membrane structure or erythrocyte metabolism. In neonates of Northern European ancestry with hemolytic jaundice in the first days of life and a negative Coombs test, Hereditary Spherocytosis (HS) or Hereditary Elliptocytosis (HE) should be considered. HS has a prevalence estimate of about one case per 2000 births and HE about one case per 3000 births. Neonates with HS tend to have a large MCHC, as shown in Fig. 103.3 [57]. When a neonate of Southeastern Asia has Coombs negative jaundice a variety of HE called Southeastern Asian Ovalocytosis should be considered. These disorders can be identified by peripheral smear, noting the distinctive abnormalities of erythrocyte shape [57–61]. All involve genetic mutations of the erythrocyte cytotoskeletal components, as noted in Table 103.2 and illustrated in Fig. 103.4.

Programs to mass-screen neonates for hemoglobinopathies appear to be successful. For instance, during a 10-year evaluation in Brussels over 100,000 neonates underwent screening for hemoglobinopathies. Sickle cell was identified in 64, six had beta-thalassemia major, four had an Hb C disease, and three had an Hb H disease [62].

Table 103.2 The more common varieties of Coombs negative neonatal hemolytic disease with abnormal erythrocyte shapes

Defect	Disorder	Proportion with this variety	Inheritance	Severity
Ankyrin	Spherocytosis	40–65%	Dominant	Mild to moderate
Band 3	Spherocytosis Southeast Asian Ovalocytosis	20–35%	Dominant	Mild to moderate
β-spectrin	Spherocytosis	15–30%	Dominant	Mild to moderate
α-spectrin	Spherocytosis	<5%	Recessive	SEVERE
Protein 4.2	Spherocytosis	<5% (Japan)	Recessive	Mild to moderate
Protein 4.1	Elliptocytosis		Dominant	Mild to moderate

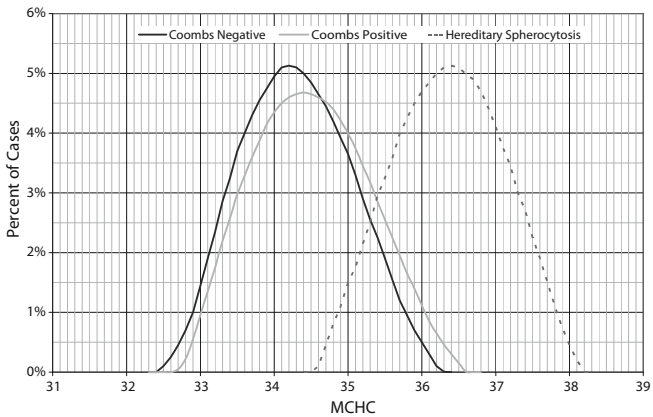

Fig. 103.3 Elevated mean corpuscular hemoglobin concentration in neonates with hereditary spherocytosis. The distribution of MCHC measurements are shown for three groups of neonates with hemolytic jaundice a). Coombs negative jaundice, b) Coombs positive jaundice, c) Hereditary spherocytosis. Reproduced from [58], with permission

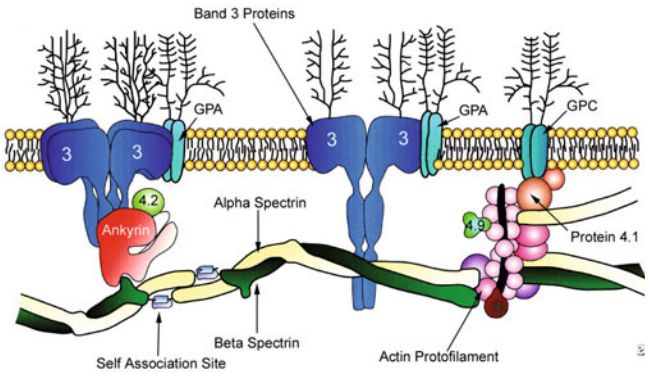

Fig. 103.4 A schematic drawing of the red cell membrane, noting the subunits described to have genetic defects as the basis for congenital abnormalities in erythrocyte shape, and predisposing to neonatal hemolytic jaundice. Reproduced from [44], with permission

103.5 Fetal and Neonatal Anemia Due to Immune-Mediated Hemolytic Disease

Various causes of fetal and neonatal hemolytic disease are noted in Table 103.3. Fetal bilirubin is cleared by the placenta and metabolized by the maternal liver [63]. As a result fetal hemolytic disease essentially never presents as severe jaundice at birth. However, after birth the products of heme catabolism must be processed by the neonate's liver, which is limited in its ability to metabolize bilirubin efficiently. This limitation is due in part to a deficiency of the cytoplasmic acceptor protein ligandin, and in part because of decreased activity of uridine diphosphoglucuronyl transferase [63, 64].

Worldwide, isoimmunization caused by maternal-fetal blood group incompatibility is the most common cause of newborn hemolytic jaundice. Antigens in the Rh, ABO, MN, Kell, Duffy, and Vel systems are well developed on fetal red cells

Table 103.3 Causes of neonatal hemolytic disease

A. Immune mediated
   1. Rh (anti-D) incompatibility
   2. ABO incompatibility
   3. Other blood group incompatibility- Duffy, Kell, Jka, MNS, Vw
   4. Maternal autoimmune hemolytic anemia

B. Erythrocytes enzyme mutations
   1. G6PD deficiency
   2. Pyruvate kinase deficiency
   3. Hexose kinase deficiency
   4. Glucose phosphate isomerase deficiency
   5. Pyrimidine 5' nucleotidase deficiency

C. Erythrocyte membrane mutations
   1. Hereditary spherocytosis
   2. Hereditary elliptocytosis/ovalocytosis (Southease Asian Ovalocytosis)
   3. Other membrane disorders

D. Infections

E. Hemoglobin defects
   1. α thalassemia
   2. γ thalassemia

F. Angiopathic hemolysis
   1. Arterovenous malformations
   2. Cavernous hemangiomas
   3. Disseminated intravascular coagulation
   4. Large vessel thrombosis
   5. Severe valvar stenosis

G. Miscellaneous causes
   1. Galactosemia
   2. Hypothyroidism
   3. Lysosomal storage diseases

during early intrauterine life [65]. They are present in the fifth to seventh gestational week and remain through the remainder of intrauterine development. Other antigens, such as the Lutheran and XgA systems, develop more slowly but are present at birth, unlike Lewis antigens, which develop after birth. By 2 years of age the red cell antigens have developed a pattern that is seen throughout the remainder of life [66]. Although A and B antigens are present early in utero, A and B isoagglutinin production occurs later during the second and third trimester [66]. By 30–34 weeks' gestation, about one-half of all fetuses have measurable anti-A or anti-B antibodies.

Alloimunization to Kell can present with unique neonatal findings. A low reticulocyte count can be seen along with a low Hb and elevated bilirubin [67]. This is because anti-Kell antibodies can bind to erythroid progenitors and reduce red cell production [68].

Extremely high titers of anti-C antibody have been associated with neonatal hemolytic disease [69]. However, routine screening of anti-C titers during pregnancy is not warranted because antibody titers do not accurately reflect the severity of hemolytic disease [70, 71].

103.6 Fetal and Neonatal Anemia due to Hemorrhage

Causes of hemorrhage that can result in fetal or neonatal anemia are noted in Table 103.4 and are divided into prenatal, perinatal, and postnatal varieties.

Table 103.4 Causes of hemorrhage in the fetus and neonate

A. Prenatal
1. Twin-twin transfusion
2. Fetal maternal hemorrhage
3. Trauma with bleeding into cord, placenta, amniotic fluid

B. Perinatal
1. Placenta previa
2. Placental abruption
3. Vasa previa
4. Velementous insertion of the umbilical cord
5. Nuchal cord
6. Trauma or incision of the cord or placenta during cesarean section
7. Rupture of the umbilical cord at delivery

C. Postnatal
1. Subgaleal hemorrhage
2. Cephalohematoma
3. Organ trauma after birth
4. Pulmonary hemorrhage
5. Intrcranial hemorrhage
6. Iatrogenic blood loss

103.6.1 Prenatal Hemorrhage

Approximately 1 pregnancy in 400 is associated with fetal to maternal hemorrhage (FMH) of 30 mL or more, and 1 pregnancy in 2000 is associated with FMH of 100 mL or more (72). FMH consisting of small volumes of blood is very common. Perhaps as many as 75% of pregnancies can be shown to have 0.01–0.1 mL of fetal blood transferred into the maternal circulation. Transfer of fetal blood cells into the mother occurs during abortions as well. This has been reported in approximately 2% of spontaneous abortions and in 4–5% of induced abortions [73]. The Kleihauer Betke stain of maternal blood evaluates the acid elution of Hb from red cells [74]. HbF resists acid elution to a greater degree than adult Hb. Therefore maternal cells appear clear (termed ghost cells), whereas any erythrocytes of fetal origin will appear pink. False positive results occur when mothers have an increase in HbF (i.e., sickle cell disease, thalassemia, and hereditary persistence of HbF). Diagnosing FMH can also be difficult to detect when the mother is blood group O and the infant is A, B, or AB, because fetal cells are rapidly cleared from the maternal circulation by maternal anti-A or anti-B antibodies and therefore they do not appear on the Kleihauer Betke stain.

Severe FMH can be suspected before delivery by decreased fetal movements and a fetal sinusoidal heart rate pattern [75, 76]. Giacoia reviewed these variables to determine if they correlated with the severity of FMH [73]. Fetal movements for a period ranging between 24 hours and 7 days were absent in 17 of 134 cases evaluated. In this group, six infants survived, five were stillborn, and five died in the neonatal period. A sinusoidal heart rate pattern was reported in 21 cases, and was associated with decreased fetal movement in 40% of the cases. No significant difference was found between the cases with a hemorrhage of less than 200 mL and those less than 200 mL. Significant FMH has been described following maternal trauma [76].

Neonates delivered after a significant FMH can be very pale, tachycardic, and tachypnec, but they generally do not have marked respiratory distress or a requirement for supplemental oxygen. Their Hb concentration can be as low as 4–6 g/dL and a significant metabolic acidosis is often present in association with poor perfusion. Other causes of pallor can be ruled out once the infant is stable. Infants with asphyxia or chronic anemia due to hemolysis can also present with pallor. These diagnoses can be distinguished from acute hemorrhage based on differences in clinical signs and symptoms. With chronic blood loss the signs of shock are usually absent. Asphyxiated infants are pale, floppy, and may have poor peripheral circulation. The Hb will be stable, but may decrease if disseminated intravascular coagulation (DIC) and internal bleeding occur.

Twin-twin transfusion is a complication of monochorionic twin gestations, occurring in 5–30% of these pregnancies [75, 76]. It involves placental anastomoses that permit transfer of blood from one twin to the other. The perinatal mortality rate can be 70% or more. About 70% of monozygous twin pregnancies have monochorionic placentas. Although vascular anastomoses are present in almost all such, not all develop

twin-twin transfusion. Acute twin-twin transfusion generally results in twins of similar size but with Hb concentrations that vary by more than 5 g/dL [75, 76]. In chronic twin-twin transfusion, the donor twin becomes progressively anemic and growth retarded, whereas the recipient twin becomes polycythemic, macrosomic, and sometimes hypertensive. Both can develop hydrops fetalis; the donor twin becomes hydropic from profound anemia, and the recipient twin from congestive heart failure and hypervolemia. The donor twin often has low amniotic fluid volumes, whereas the recipient twin has increased amniotic fluid, due to significant differences in blood volume, renal blood flow, and urine output.

Chronic twin-twin transfusion can be diagnosed by serial prenatal ultrasound measuring cardiomegaly, discordant amniotic fluid production, and fetal growth discrepancy of >20%. Percutaneous umbilical blood sampling can determine if Hb concentration differences of greater than 5 gm/dL exist. After birth, the donor twin may require transfusions and can have neutropenia, hydrops from severe anemia, growth retardation, congestive heart failure, and hypoglycemia. The recipient twin is often the sicker of the two, with problems including hypertrophic cardiomyopathy, congestive heart failure, polycythemia, hyperviscosity, respiratory difficulties, hypocalcemia, and hypoglycemia. Neurologic evaluation and imaging are imperative because the risk of antenatally acquired neurologic cerebral lesions is 20–30% in both twins. The incidence of neurologic morbidity following the intrauterine death of one of the fetuses averages 20–25%. Morbidities include multiple cerebral infarctions, hypoperfusion syndromes from hypotension, and periventricular leukomalacia. Long-term neurologic follow-up is indicated for all survivors of twin-twin transfusion [75, 76].

Prenatal treatment for twin-twin transfusion consists of close monitoring and reduction amniocenteses to decrease uterine stretch and prolong the pregnancy. Selective feticide of the hydropic twin has been advocated by some and has resulted in the survival of the healthier twin in some studies [77]. Treatment in utero has occurred during some pregnancies using laser ablation of bridging vessels, resulting in improved survival rates up to around 50%, with approximately 70% of the pregnancies having at least one survivor [78–80]. However, the survival rate without morbidity in the surviving twin is approximately 50%. Supski et al performed a meta analysis of 140 cases to correlate types of treatment with outcome [81]. They found no differences in outcome between amnioreduction, fetoscopy, septostomy, or close observation.

103.6.2 Perinatal Hemorrhage

Perinatal blood loss to the fetus can occur with various obstetric complications, such as placenta previa, placental abruption, incision or tearing of the placenta during cesarean section, and cord evulsion. When a fetus undergoes significant blood loss back into the placenta the term fetoplacental hemorrhage is used. Placental anomalies such as a multilobed placenta and placental chorioangiomas can be a source of perinatal bleeding [82].

Placental abruption occurs in 3–6 per 1000 live births. Risk factors of placental abruption include prolonged rupture of the membranes, severe fetal growth restriction, chorioamnionitis, hypertension, maternal diabetes, cigarette smoking, and advanced maternal age [83]. The incidence of abruption increases with lower gestational age. Neonatal mortality rates from abruption range from 0.8–2.0 per thousand births, or 15–20% of the deliveries in which significant abruption occurs.

Women with a history of a previous cesarean birth and increased parity are at increased risk of placenta previa [84], a condition where part or all of the placenta overlies the cervical os. Cigarette smoking is associated with a 2.6- to 4.4-fold increased risk of placenta previa [85]. Prenatal diagnosis of vasa previa (anomalous vessels overlying the internal os of the cervix) can be made with transvaginal color Doppler, and should be suspected in cases of antepartum or intrapartum hemorrhage. Although uncommon (1 in 3000 deliveries), the perinatal death rate is high, ranging from 33–100% when this condition is undetected before delivery [86].

Neonates delivered after placental abruption or after placenta previa can be anemic but they can also have signs of hypoxia and ischemia. The majority of blood lost in an abruption or previa is maternal blood, but the neonate can have some degree of anemia as well. Therefore, when perinatal blood loss is recognized or suspected, the neonate's Hb should be measured at birth and again 12 hours or so later. A Kleihauer Betke stain can be performed on maternal blood to determine if fetal hemorrhage can be documented. Monitoring bleeding mothers with ultrasound might detect placental abnormalities.

Cord rupture due to traction on a shortened or abnormal umbilical cord usually occurs on the fetal side. Cord aneurysms, varices, and cysts can all lead to a weakened cord. Cord infections (funisitis) can also weaken the cord and increase the risk of rupture. Infants born precipitously may be at increased risk for hemorrhage due to a ruptured cord.

Cord hematomas occur infrequently (1 in 5000–6000 deliveries) and can be a cause of fetal blood loss and perinatal mortality. Intrauterine death can occur due to compression of the umbilical vessels by a cord hematoma. Cord hematomas can result from trauma from percutaneous umbilical blood sampling. Hematomas of the cord can be diagnosed in utero by ultrasound [86, 87].

Subamniotic hematomas can occur when chorionic vessels rupture near the cord insertion. Most subamniotic hematomas are the result of traction on a normal or shortened umbilical cord and are not noted until after delivery.

Velamentous insertion of the umbilical cord occurs when the umbilical cord enters the membranes distant from the placenta. This is present in 0.5–2.0% of pregnancies [88]. Blood vessels left unprotected by Wharton jelly are more likely to tear. Rupture of anomalous vessels in the absence of traction or trauma can occur even if the cord itself attaches

centrally or paracentrally. Fetal mortality remains very high in this condition, often because detection by routine ultrasound is rare [89].

103.6.3 Postnatal Hemorrhage

Loss of fetal blood into the placenta can occur during delivery. In fact, a net shift of blood from the fetus into the placenta is a rather common cause of low-grade neonatal anemia. At term, the fetal-placental-umbilical cord unit contains about 120 mL of blood per kg body weight. After delivery, but before the umbilical cord is severed, blood in this unit can flow predominantly toward or away from the neonate. A fetoplacental hemorrhage can occur when the neonate is held significantly higher than the placenta after birth. Also, neonates can lose up to 20% of their blood volume when born with a tight nuchal cord, which allows blood to be pumped through umbilical arteries toward the placenta, while constricting flow back from the placenta to the baby, through the umbilical vein, which is more easily constricted due to its thin wall.

As shown in Fig. 103.5, blood loss can occur into the subgaleal space before or after birth. This is seen most commonly with difficult deliveries requiring vacuum or forceps assistance. Subgaleal hemorrhages are potentially life-threatening and must be recognized as early as possible to prevent significant morbidity or mortality. The hemorrhage occurs when bridging veins are torn, allowing blood to accumulate in the large potential space between the galea aponeurotica and the periosteum of the skull. The subgaleal space extends from the orbital ridge to the base of the skull and can accommodate a volume equivalent to a neonate's entire blood volume [90, 91].

Subgaleal hematomas can form because of risk factors such as coagulopathy or asphyxia, but vacuum extraction itself is a risk factor for their development. The diagnosis should be considered in the presence of a ballotable fluid collection in dependent regions of the infant's head, coupled with signs of hypovolemia [92]. Treatment requires restoration of blood volume and control of bleeding. Exsanguination due to subgaleal hemorrhage has been reported. A suggested way to estimate the volume of blood lost is by following head circumference; 40 mL of blood has been lost for every 1 cm increase in head circumference that occurs [93]. The duration of vacuum application is thought to be the best predictor of scalp injury, followed by duration of second stage of labor and paramedian cup placement. Of those with reported subgaleal hemorrhages, 80–90% had some history of vacuum or instrument-assisted delivery [92, 93]. Limiting the frequency and duration of vacuum assistance in high-risk infants might decrease the incidence of subgaleal hematomas.

Anemia appearing after the first 24 hours of life in a nonjaundiced infant can be a sign of hemorrhage. Hemorrhages

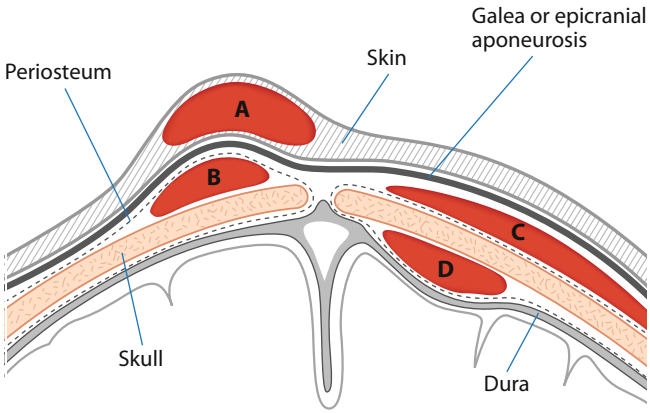

Fig. 103.5 A schematic drawing of the scalp and skull of a newborn infant, illustrating four distinct anatomic spaces where fluid or blood might collect during or after delivery. **A** caput hemorrhage, **B** cephalhematoma, **C** subgaleal hemorrhage, **D** extradural hemorrhage

can be visible, such as a cephalohematoma, or occult. Breech deliveries can be associated with renal, adrenal, or splenic hemorrhage into the retroperitoneal space. Delivery of macrosomic infants, such as infants born to diabetic mothers, can result in hemorrhage. Infants with overwhelming sepsis can bleed into soft tissue and organs.

In addition to causing anemia, adrenal hemorrhage can result in circulatory collapse due to the loss of organ function. The incidence of adrenal hemorrhage is 1.7 per 1000 births [94]. Adrenal hemorrhage can also affect surrounding organs. Intestinal obstruction and kidney dysfunction have been reported in infants with adrenal hemorrhage [95]. The diagnosis can be made using ultrasonography, during which calcifications or cystic masses are noted. Adrenal hemorrhage can be distinguished from renal vein thrombosis (RVT) by ultrasound, in that RVT generally results in a solid mass. Occasionally, both entities coexist in the same patient.

The liver of a neonate is prone to iatrogenic rupture, resulting in high morbidity and mortality [96]. Neonates with this problem can appear asymptomatic until the liver ruptures and hemoperitoneum occurs. This problem can occur in both term and preterm infants [97] and has been associated with chest compressions during cardiopulmonary resuscitation. Surgical intervention has been reported to save some infants but the mortality is very high [97].

Splenic rupture can result from birth trauma or as a result of distention caused by extramedullary hematopoiesis, such as that seen in erythroblastosis fetalis. Abdominal distension and discoloration, scrotal swelling, and pallor are clinical signs of splenic rupture; these can also be seen with adrenal or hepatic hemorrhage [96–98].

More rare causes of hemorrhage in the newborn period include hemangiomas of the gastrointestinal tract [99], vascular malformations of the skin, and hemorrhage into soft tumors, such as giant sacrococcygeal teratomas. Occult intraabdominal hemorrhage can occur with fetal ovarian cysts, which are

usually benign and resolve spontaneously. One case of fetal anemia was diagnosed by a spontaneous hemorrhage into a fetal ovarian cyst and was managed by intrauterine blood transfusions [100].

103.7 Fetal and Neonatal Anemia Due to Congenital Infection

Neonatal bacterial sepsis can cause anemia on the basis of hemolysis, DIC, and hemorrhage. Some microorganisms responsible for neonatal sepsis produce hemolytic endotoxins that result in accelerated erythrocyte destruction, often associated with a microangiopathic process [101].

Congenital viral infections can also cause hemolytic anemia. Congenital syphilis can present with hemolytic anemia. Initial maternal screening for syphilis can be negative despite overwhelming infection, a condition termed the prozone effect [101], which occurs when a higher than optimal amount of antibody in the tested sera prevents the flocculation reaction seen in positive reagin test. In cases of nonimmune hydrops, nontreponemal testing should be repeated using serum dilutions to prevent a missed diagnosis of syphilis in women with negative syphilis serologic results.

Fetal and neonatal infection with parvovirus B19 can cause severe anemia, hydrops, and fetal demise [102]. This is generally a hypoplastic anemia, but hemolysis can occur as well. The virus replicates in erythroid progenitor cells and results in red cell aplasia. In utero transfusions for hydropic fetuses can be successful. Intrauterine fetal infusion of B19 IgG-rich high titer gamma globulin has been reported to be successful [103].

Other fetal infections associated with neonatal anemia include malaria and HIV. Congenital malaria is seen rarely in the USA, generally in large cities where imported cases of malaria are increasing. In certain African countries, congenital malaria has been reported in up to 20% of neonates [104]. Congenital HIV infection in a neonate is generally asymptomatic. However infants born to mothers on zidovudine can have a hypoplastic anemia due to suppressive effects of the drug on fetal erythropoiesis [105].

103.8 Anemia of Prematurity and Other Hypoproliferative Disorders

Impaired erythrocyte production can occur in a fetus or neonate for a variety of reasons. Lack of an appropriate or sufficient marrow environment (as seen in osteopetrosis), lack of specific substrates or their carriers (e.g., iron, folate, vitamin B12, or transcobalamin II deficiency) and lack of specific growth factors (e.g., decreased Epo production or abnormalities in Epo receptors) can be causative.

103.8.1 Anemia of Prematurity

Infants delivered before 32 completed weeks gestation typically develop a transient and unique anemia knows as the anemia of prematurity. During the first week or two after birth, while in an intensive care unit, anemia secondary to phlebotomy loss is common. However, after this period has passed, a second anemia is sometimes seen; characterized as a normocytic, normochromic, hyporegenerative anemia, with serum Epo concentrations significantly below those found in adults with similar degrees of anemia [106]. This anemia is not responsive to the administration of iron, folate, or vitamin E. Some infants with the anemia of prematurity are asymptomatic, whereas others have clear signs of anemia that are alleviated by erythrocyte transfusion. These signs include tachycardia, rapid tiring with nipple feedings, poor weight gain, increased requirements for supplemental oxygen, episodes of apnea and bradycardia, and elevated serum lactate concentrations.

The reason preterm infants do not significantly increase serum Epo concentration during this anemia is not known. Indeed, it is unclear whether production of Epo does in fact increase, yet the serum concentration does not. Certainly their erythroid progenitors are sensitive to Epo [107, 108], and concentrations of other erythropoietic growth factors appear to be normal [109].

The molecular and cellular mechanisms responsible for the anemia of prematurity remain undefined. Some explanations include the transition from fetal to adult Hb, shortened erythrocyte survival, and hemodilution associated with a rapidly increasing body mass [110]. It is unknown whether preterm infants rely on Epo produced by the liver (the source of Epo in utero), or that produced by the kidney, or a combination of the two. Regardless of the mechanism responsible for the anemia of prematurity, exogenous Epo administered to preterm infants accelerates effective erythropoiesis [111]. A meta-analysis of studies evaluating the use of "late" Epo administration to prevent and treat the anemia of prematurity reveals a positive effect on decreasing transfusion requirements in preterm infants [112]. In addition, beneficial neurodevelopmental effects of recombinant Epo administration have been reported in preterm infants [113–116].

Pharmacokinetic studies of darbepoetin, the long-acting erythropoietic stimulator, have been conducted among neonates with the anemia of prematurity, with the speculation that less frequent dosing and cost savings might render Darbepoetin a more attractive alternative than recombinant Epo

Table 103.5 Terminal half-life of darbepoetin among adults, children, and neonates after subcutaneous or intravenous dosing

	After subcutaneous dosing	After intravenous dosing
Adults	49 hours	25 hours
Children	43 hours	22 hours
Neonates	22 hours	10 hours

for treating the anemia of prematurity [117–119]. Following subcutaneous and intravenous dosing, Darbepoetin has a considerably shorter terminal half-life in neonates than in adults (Table 103.5). Intravenous dosing appears to be as effective as subcutaneous dosing.

103.8.2 Other Hypoproliferative Anemias and Associated Syndromes

During the neonatal period hypoproliferative anemias are rare, with the exception of the anemia of prematurity, which is common (Table 103.6). Diamond-Blackfan syndrome can be diagnosed at birth but usually is not recognized until after 2–3 months of age. At least 10–25% of infants with Diamond-Blackfan syndrome have anemia at birth [120, 121], and severe anemia with hydrops has been reported. Aase syndrome, another congenital hypoplastic anemia syndrome involving skeletal anomalies [122], is sometimes classified as a variant of Diamond-Blackfan syndrome. Congenital dyserythropoietic anemia is a rare disorder marked by ineffective erythropoiesis, megaloblastic anemia, and characteristic abnormalities of the nuclear membrane and cytoplasm seen on electron microscopy. Fanconi anemia almost never manifests during the neonatal period. This autosomal-recessive disorder is characterized by marrow failure and congenital anomalies, including abnormalities in skin pigmentation, gastrointestinal anomalies, renal anomalies, and upper limb anomalies [123].

Osteopetrosis involves osteoclast dysfunction, resulting in a decreased marrow space [124, 125]. Developmental delay, ocular involvement, and neurodegenerative findings occur in these patients in association with hypoplastic anemia. Patients are generally treated with stem cell transplantation, but they are particularly susceptible to post-transplantation complications after myeloablation, and reduced-intensity conditioning programs may be helpful.

Pearson syndrome is a congenital hyporegenerative anemia that can progress to pancytopenia, and additionally affects the exocrine pancreas, liver, and kidneys [126]. These patients can present during the neonatal period, but typically do so later in infancy. Features include failure to thrive and cytopenia. The marrow examination shows characteristic vacuoles within erythroid and myeloid precursors, hemosiderosis, and ringed sideroblasts. The syndrome is caused by a loss of large segments of mitochondrial DNA [127, 128].

Table 103.6 Syndromes associated with congenital anemia

Syndrome	Phenotypic features	Genotypic features
Adenosine deaminase deficiency	Autoimmune hemolytic anemia, reduced erythrocyte adenosine deaminase activity	AR, 20q13.11
Congenital dyserythropoietic anemias	Type I (rare): megaloblastoid erythroid hyperplasia and nuclear chromatin bridges between nuclei; type II (most common): "hereditary erythroblastic multinuclearity, positive acidified serum (HEMPAS) test, increased lysis to anti-i; type III: erythroblastic multinuclearity ("gigantoblasts"), macrocytosis	Type I: 15q15.1-q15.3; type II: 20q11.2; type III: 15q21
Diamond-Blackfan syndrome	Steroid-responsive hypoplastic anemia, often macrocytic after 5 months of age	AR; sporadic mutations and AD inheritance described; 19q13.2, 8p23.3-p22
Dyskeratosis congenita	Hypoproliferative anemia usually presenting between 5–15 yr of age	X-linked recessive, locus on Xq28; some cases with AD inheritance
Fanconi pancytopenia	Steroid-responsive hypoplastic anemia, reticulocytopenia, some macrocytic RBCs, shortened RBC lifespan. Cells are hypersensitive to DNA cross-linking agents	AR, multiple genes: complementation; group A: 16q24.3; B:; C: 9q22.3; D2: 3p25.3; E: 6p22-p21; F: 11p15; G: 9p13
Osler hemorrhagic telangiectasia syndrome	Hemorrhagic anemia	AD, 9q34.1
Osteopetrosis	Hypoplastic anemia from marrow compression; extramedullary erythropoiesis.	AR: 16p13, 11q13.4-q13.5; AD: 1p21; lethal: reduced osteoclasts
Pearson syndrome	Hypoplastic sideroblastic anemia, marrow cell vacuolization	Pleioplasmatic rearrangement of mitochondrial DNA; X-linked or AR
Peutz-Jeghers syndrome	Iron deficiency anemia from chronic blood loss	AD, 19p13.3
X-linked α-thalassemia/ mental retardation (ATR-X and ATR-16) syndromes	ATR-X: hypochromic, microcytic anemia; mild form of hemoglobin H disease ATR-16: more significant hemoglobin H disease and anemia are present	ATR-X: X-linked recessive, Xq13.3; ATR-16: 16p13.3, deletions of α-globin locus

AD autosomal dominant, *AR* autosomal recessive, *RBC* red blood cell.

103.9 Considerations Regarding Erythrocyte Transfusion in the Neonatal Period

Best practices in neonatal transfusion medicine are largely undefined. A very basic issue that remains unsettled is at what level to keep the Hb concentration during the NICU stay. Specifically, it is not clear whether to keep a NICU patient's Hb as high as it would be in utero (often requiring multiple transfusions to do so) or whether to permit the Hb to fall to considerably lower values, attempting to avoid or minimize transfusions. Attempts have been made to define the best Hb range for NICU patients, but study findings are discordant.

In a single-centered study, Bell et al randomized 100 neonates with birth weights <1300 g (average birth weight 956 grams) to maintain a hematocrit in a higher range versus a lower range. Those kept in the lower range received fewer transfusions (average of 2 additional transfusions per patient), but were more likely to have intraparenchymal brain hemorrhage or periventircular leukomalacia [129]. In contrast, the PINT study (Premature Infants in Need of Transfusion), a larger multicentered study involving 451 extremely low birth weight neonates (average birth weight 770 grams), concluded that neonates randomized to the lower hematocrit range had fewer transfusions but similar neurodevelopmental outcomes [130].

Various neonatal transfusion guidelines have been used over the last 2 decades, and research is ongoing to determine the optimal strategy for administering red cell transfusions to preterm and term neonates. One strategy developed by the Canadian Paediatric Society in 2002 is shown in Table 103.7. When considering a transfusion in a preterm infant with a low hematocrit which is not due to acute hemorrhage, it should be determined if the infant needs an immediate increase in oxygen to tissues. If so, then treatment consists of a transfusion of packed red cells. If there is no evidence that an immediate increase in oxygen delivery is necessary, then treatment with red cell growth factors and appropriate substrates might be considered. As the process of stimulating erythropoiesis requires at least a week to significantly impact the reticulocyte count, and may not appreciably increase the hemoglobin concentration during that time, the infant should continue to be observed for signs consistent with anemia.

A method of reducing erythrocyte transfusions, among a subset of preterm neonatal patients, is to begin the administration of recombinant Epo to those with low hematocrits after the first three weeks of life [110–113]. Recombinant Epo certainly stimulates erythropoiesis in such patients, although its combination with additional folate, iron, vitamin E, and vitamin B12 may be superior to recombinant Epo alone [132].

Haiden and colleagues achieved significantly greater success in preterm infants <800 grams (38% of infants not transfused) when vitamin B12 at a dose of 21 mg/kg/week SC was added to a regimen of Epo, iron, vitamin E and folate [132]. When combined with limited phlebotomy losses, this therapy shows great promise in ELBW infants.

Another method of reducing erythrocyte transfusions to ill neonates is to delay clamping the umbilical cord. Even a delay of 30 seconds can result in improved iron status [133], fewer transfusions [130], and perhaps superior neurodevelopmental outcomes [134-137].

Yet another method of reducing or postponing early erythrocyte transfusions among extremely low birth weight neonates is to draw the blood for the initial laboratory tests from the placenta not from the neonate. The initial phlebotomy of an extremely low birth weight neonate, on admission to the NICU, can include a blood culture, CBC, type and cross-match, metabolic screen, blood gas, electrolytes, and glucose. Sometimes other studies such as coagulation tests are also drawn at or shortly following NICU admission. The total blood volume needed for these base-line NICU laboratory studies can be 4–5 mL or more. In a 400–500 gram neonate this can exceed 10% of the total blood volume. Early transfusions in the NICU can also be reduced by careful attention to phlebotomy volumes during the first days following delivery. Transfusions given during the first week or two are principally to replace phlebotomy losses for laboratory tests. Employing laboratory methods that minimize blood loss will reduce early transfusions. Such methods include point of care monitors, point of care analyzers, and a concerted effort to use the smallest amount of blood possible for the needed laboratory studies [137–138].

Table 103.7 Canadian Paediatric Society recommendations for RBC transfusions in newborn infants

- Acute blood loss resulting in hypovolemic shock
- Hemoglobin between 10 and 12 g/dL or hematocrit between 30 and 35% in extreme illness conditions for which RBC transfusion may improve oxygen delivery to vital organs
- Hemoglobin between 6 and 10 g/dL or hematocrit between 20% and 30%, in severely ill neonates and/or on mechanical ventilation with compromised oxygen delivery
- Hemoglobin below 6 g/dL or hematocrit below 20% with absolute reticulocyte count 100–150 × 10^3/μL or less, suggesting low plasma concentration of erythropoietin, with the presence of the following clinical signs: poor weight gain, heart rate >180 beats/minute, respiratory distress and increased oxygen needs, and lethargy

Modified from [131].

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Fetal and Neonatal Hydrops

Gennaro Vetrano and Mario De Curtis

104.1 Introduction

Hydrops fetalis (i.e., fetal hydrops) (HF) is a serious condition defined as an abnormal accumulation of fluid in two or more fetal compartments. It presents as ascites, pleural effusion, pericardial effusion and skin edema. In some patients, it may also be associated with polyhydramnios and placental edema. In 1943, Potter was the first to distinguish non-immune hydrops fetalis (NIHF) from immune hydrops [1].

104.2 Epidemiology

Previously, hemolytic disease due to Rh incompatibility was the main cause of both fetal and neonatal immune hydrops. These days approximately 90% of cases of hydrops fetalis are due to non-immune disease, with the number of liveborn ranging from 1:1500 to 1:3800 [2, 3]. Hydrops fetalis is much more common in Southeast Asia; in Thailand the expected frequency of non-immune hydrops fetalis due to homozygous alpha-thalassemia or Bart hydrops ranges from one every 500 to one every 1500 pregnancies [4, 5]. Although the availability of ultrasound technology has greatly improved antenatal diagnosis of HF, perinatal mortality (PNM) remains high.

104.3 Pathogenesis

The basic mechanism for the formation of HF is an imbalance between interstitial fluid production and lymphatic return. Fluid accumulation in the fetus can be due to (a) heart failure, (b) anemia, (c) obstructed lymphatic flow, or (d) decreased plasma osmotic pressure. The fetus is particularly susceptible to interstitial fluid accumulation because of its greater capillary permeability, compliant interstitial compartments, and liability for raised venous pressure because of impaired lymphatic return [5, 6]. Clinical and animal studies have shown that elevation of central venous pressure (CVP) has a pivotal role in the development of fetal hydrops [7]. Increased CVP causes edema and effusions by increasing capillary hydrostatic pressure and decreasing lymphatic return [8]. Albumin is the main oncotically active plasma protein and when its hepatic synthesis is impaired, transcapillary fluid movements increase [5, 6]. Hypoproteinemia and hypoalbuminemia are common in human hydrops. However, studies in humans and animals have shown that hypoalbuminemia is unlikely to trigger this condition [9].

104.4 Etiology

The possible etiologies of HF are still being unrevealed, as previously unknown causes are reported. However, despite extensive pre- and postnatal investigations, no definite cause can be determined in 33% of cases [10]. HF is an end-stage process and a non-specific finding that is associated with a range of abnormalities. Its causes can be divided into 6 broad categories: hematological disorders, cardiovascular and infectious conditions, genetic abnormalities, tumors, and idiopathic.

Table 104.1 summarizes the causes of fetal hydrops.

104.5 Diagnosis

A pregnant woman with polydramnios, severe anemia, toxemia or isoimmune disease should undergo further investigation.

The antenatal diagnosis of HF is made by the ultrasound finding of fluid accumulation in the fetus or placenta. Specifically, excess serous fluid should be identified in at least one

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Table 104.1 Causes of hydrops fetalis

Hematological
- Isoimmunization (immune hydrops) (hemolytic disease of the newborn, erythroblastosis)
  - Rh (most commonly D; also C, c, E, e)
  - Kell
  - ABO
  - Others
- Other hemolytic disorders
  - Glucose phosphate isomerase deficiency
  - Pyruvate kinase deficiency
  - G-6-PD deficiency
- Disorders of red cell production
  - Diamond-Blackfan syndrome
  - Leukemia (usually associated with Down or Noonan syndrome)
  - Alpha-thalassemia (Bart hemoglobinopathy)
  - Parvovirus B19
  - Others
- Fetal hemorrhage
  - Placental subchorial tumors
  - Feto-maternal hemorrhage
  - Twin-to-twin transfusion
  - Isoimmune fetal thrombocytopenia
  - Others

Cardiovascular
- Structural anomalies
  - Abnormalities of left ventricular outflow
  - Abnormalities of right ventricular outflow
  - Other vascular malformations
- Non-structural anomalies
  - Obstruction of venous return
  - Supraventricular tachycardia
  - Congenital heart block
  - Prenatal closure of the foramen ovale or ductus arteriosus
  - Myocarditis
  - Idiopathic arterial calcification or hypercalcemia

Infectious
- Parvovirus B19
- Cytomegalovirus (CMV)
- Syphilis
- Herpes simplex
- Toxoplasmosis
- Hepatitis B
- Adenovirus
- Ureaplasma urealyticum
- Coxsackie virus type B
- Listeria monocytogenes

Genetic
- Inborn errors of metabolism
  - Glycogen-storage disease, type IV
  - Lysosomal storage diseases
  - Hypothyroidism and hyperthyroidism
  - Others
- Genetic syndromes
- Chromosomal syndromes
  - Beckwith-Wiedemann syndrome (trisomy 11p15)
  - Cri-du-chat syndrome (chromosomes 4 and 5)
  - Trisomy 10, mosaic
  - Trisomy 13
  - Trisomy 15
  - Trisomy 18
  - Trisomy 21 (Down syndrome)
  - Turner syndrome (45, X)
  - Others

Tumors and others
- Intrathoracic tumors or masses
- Abdominal tumors or masses
- Other conditions
  - Placental choriocarcinoma
  - Placental chorangioma
  - Cystic hygroma
  - Intussusception
  - Meconium peritonitis
  - Intracranial teratoma
  - Sacrococcygeal teratoma

Idiopathic

space (ascites, pleural effusion, or pericardial effusion), accompanied by skin edema (> 5 mm thick), or fluid in two potential spaces without edema [11, 12]. Ascites can be detected when a minimum of 50 mL is present in the fetal abdomen [13]. Polyhydramnios and placental thickening (> 6 cm thick) may be present, but oligohydramnios is a particularly ominous finding when it develops in non-immune hydrops fetalis [14].

The subsequent workup of the hydropic fetus should focus on detecting the underlying cause. In general, the first step is to collect detailed information about the mother's medical history, specifically in relation to hereditary or metabolic diseases, diabetes, anemia, exposure to infectious agents, and use of medication. The second step includes a detailed ultrasound examination of the fetus and maternal investigations. The third step, after obtaining maternal results, is a systematic approach to the fetus, including invasive testing, such as villocentesis, amniocentesis, cordocentesis and sampling of any effusions. Invasive investigations of the fetus are necessary when maternal bloods and ultrasound examination fail to provide a definitive cause of HF. The recommended workup of a fetus with HF is shown in Table 104.2.

If the etiology of HF is not identified before birth, postnatal investigations should be done. Blood samples for laboratory analysis are similar to those taken antenatally: blood group including Rh status, direct Coombs antibody screen, full blood cell count, karyotype, metabolic and chemistry studies, hemoglobin electrophoresis, if indicated. Structural defects should be evaluated using skeletal radiographs and ultrasound. A genetic consultation may also be helpful, particularly to determine risk of recurrence. In case of intrauterine or neonatal death, an autopsy is mandatory.

104.6 Treatment

The management of hydrops fetalis is a great challenge for fetal medicine specialists and neonatologists.

A woman with a hydropic fetus should be hospitalized in a level 3 perinatal centre if antenatal non-stress testing (NST)

Table 104.2 Antenatal evaluation of hydrops fetalis

Maternal history
- Age, parity, gestation
- Hereditary or metabolic diseases, anemia
- Recent infections or contacts
- Medication use

Maternal laboratory evaluation
- Complete blood cell count
- Blood type, Rh, indirect Coombs antibody screen
- Kleihauer-Betke stain
- Syphilis, TORCH and parvovirus B19 titers
- Culture for group B streptococcus, Listeria
- Maternal triple screen
- Oral glucose tolerance test
- Optional, as indicated:
  - Metabolic studies
  - Hemoglobin electrophoresis
  - G6PD, pyruvate kinase
  - Autoimmune screen (SLE, anti-Ro and -La)

Ultrasonography
- Identify anatomic abnormalities
- Evaluate extent of edema and effusions
- Exclude twin pregnancy
- Doppler blood flow studies

Fetal echocardiography
- Look for cardiac malformation, arrhythmia

Amniocentesis
- Karyotype
- Culture or PCR for TORCH, parvovirus
- Amniotic fluid alpha-fetoprotein
- Restriction endonucleases (thalassemias)
- Lecithin-sphingomyelin ratio, phosphatidyl glycerol to evaluate lung maturity

Fetal blood sampling
- Karyotype
- Complete blood cell count
- Blood type; hemoglobin electrophoresis
- Blood chemistry, albumin, gases
- Culture or PCR for TORCH, parvovirus
- Metabolic testing (Tay-Sacks, Gaucher, GM_1 gangliosidosis)

Fetal effusion sampling
- Culture or PCR for TORCH, parvovirus
- Protein content
- Cell count and cytology

G6PD glucose-6-phosphate dehydrogenase, *PCR* polymerase chain reaction, *SLE* systemic lupus erythematosus, *TORCH* toxoplasmosis, other agents, rubella, cytomegalovirus, herpes simplex. Modified from [10].

Table 104.3 Conservative management in hospital

- Hospitalize the patient in the presence of
  - Fetal skin thickening
  - Pericardial effusion
  - Nonreactive NST
  - Biophysical profile (BPP) ≤ 6
  - Subjective decreased fetal movement
  - Gestational age below 32–34 weeks
- Treat the underlying cause, if possible
- Administer antenatal corticosteroids
- Monitor serial growth and effusion volumes
- NST and BPP every 2–3 days

NST non-stress test.

[15] and biophysical profile (BPP) [16] are not reassuring (Table 104.3). At the same time, efforts should be continued to determine the underlying etiology of HF. Delivery is indicated after 34 weeks' gestation, or earlier if there is evidence of a mature fetal lung profile at amniocentesis or if the fetal condition deteriorates. Delivery is also necessary in cases of compromised maternal conditions due to the mirror syndrome (maternal hydrops) [17], a pre-eclampsia-like disease in mothers of hydropic fetuses.

The appropriate treatment of the fetus with hydrops, which carries a high mortality, can only be undertaken after a precise and detailed diagnosis. Full parental involvement is essential because the associated abnormalities may be severely debilitating or even lethal. In addition, invasive fetal treatment and elective preterm delivery remain controversial. Therefore obstetricians, fetal medicine specialists and neonatologists should consult about the optimal timing of delivery also involving pediatric surgeons, cardiologists, and cardiothoracic surgeons. Various anecdotal approaches are found in the literature, but no properly designed clinical trials have been done to provide the clinician with an evidence-base for management. Furthermore, the hydropic process may resolve spontaneously. Thus, management schemes aim to correct the underlying pathophysiology, including fetal transfusion to correct anemia (regardless of the cause), drug treatments for cardiac arrhythmias, correction or reduction of space-occupying lesions that impede cardiac venous or lymphatic return, and procedures intended to stop fetal blood loss (regardless of cause) [18–20].

Fetal transfusion with packed red blood cells (RBCs) given intraperitoneally has become accepted as standard care for the fetus with severe anemia. It carries low risk, even if there is no definitive evidence from randomized clinical trials. This approach has been used successfully in the treatment of severely anemic fetuses of isoimmunized pregnancies and to correct anemia due to various other causes. More recently, other routes (percutaneous umbilical vein, intrahepatic umbilical vein, umbilical artery, intracardiac transfusions) for the administration of blood products to the fetus have been reported. Other approaches have been aimed at the mother, fetus and newborn baby. Maternal plasmapheresis, promethazine or corticosteroids have been used for the mother. Fetal therapies have included partial packed-cell exchange transfusion, fetal intravenous IgG, platelet transfusion, and the administration of human granulocyte-stimulating factor. Neonatal stem cell transplantation has been used for α-thalassemia [21]. However, these newer therapeutic techniques have a greater risk to the fetus than the intraperitoneal route and should therefore be used cautiously.

Highly vascular tumor masses and acute, massive twin-to-twin hemorrhages are life-threatening diseases that may justify life-threatening treatment. Techniques such as tumor debulking surgery, surgery for active bleeding, photocoagulation

and radiofrequency thermal ablation may all be helpful in the treatment of fetal conditions such as sacrococcygeal tumors, highly vascularised fetal intraabdominal, thoracic, or placental masses, and when there is massive arteriovenous shunting [22–23].

The management of the twin-to-twin transfusion syndrome is currently an unresolved problem: treating an anemic fetus with transfusions has shown no evidence of benefit; volume reduction for the transfusion recipient or a combination of transfusion and fetal reduction has rarely been used or may not correct the ongoing pathophysiology. Furthermore, feticide of the affected twin is often followed by the development of hydrops in the previously normal surviving twin [24].

Treatments of fetal arrhythmias include doing nothing, drugs, and immediate delivery. In the presence of fetal maturity, the simplest and most direct approach is delivery of the affected fetus and treatment of the arrhythmia directly after birth. If this is not possible, drugs have been used. Drugs have been administered to mother, or fetus, or both. Medications have included digitalis, furosemide, flecainide, verapamil, amiodarone, propranolol, procainamide, quinidine, adenosine, sotalol, terbutaline, corticosteroids, and immunoglobulins. Various drug combinations have also been used. However, the choice of the drug remains empirical and arbitrary, until definitive evidence from clinical trials becomes available [25, 26].

The management of space-occupying masses varies depending on the type of lesion and from centre to centre. If immediate delivery is not practicable, the mass is either reduced or removed. Pleural and pericardial effusions and ascites have been treated with single or serial drainage. Fetal surgery with definitive correction of the underlying anomaly has also been used. Successes and failures have been reported with all methods; there is no evidence suggesting that one approach is better than another [27].

Postnatal management of HF poses a unique set of problems for the neonatologist. Treatment of the infant after delivery is helped by knowing the cause. In addition to appropriate equipment and supplies for resuscitation, a skilled team of health care professionals (neonatologists, nurses, respiratory therapists, radiograph and ultrasonography technicians) should be present in the delivery room [28, 29]. Fluid in the pleural, pericardial and abdominal cavities may require aspiration in the delivery room to allow adequate ventilation and circulation. Umbilical arterial and venous catheters are sited to monitor and treat arterial pressure, blood gases, venous pressure, hematocrit and the metabolic state of the infant. Packed red cells or whole blood cross matched with the mother should be available for the correction of severe anemia by partial exchange transfusion, even when due to non-immune causes. Surfactant therapy and mechanical ventilation are used to manage surfactant deficiency and pulmonary hypoplasia, which may be associated with hydrops. Fluid intake is based on an estimate of the infant's "dry weight" (e.g., 50th percentile for gestational age) and kept to a minimum (e.g., 40–60 mL/kg/day) until the edema is resolved. Inotropic support (e.g., dopamine) may be required to improve cardiac output [30, 31].

104.7 Prognosis

Estimates of mortality vary widely. The condition has a mortality rate of virtually 100% when structural defects are present or the cause of HF is unknown. Most case series report 60–90% mortality, although notable improvements have been described in more recent reports [5]. In cases of tachyarrhythmias, the prognosis has been improved by antenatal antiarrhythmic treatment. Cases presenting before 24 weeks have a worse prognosis [32], whereas those that present later may benefit from delivery and intensive neonatal care. The risk of recurrent hydrops in a subsequent pregnancy is low, although one series reported a 10% risk of recurrence [18]. In conclusion, despite the fetus with hydrops having profoundly compromised perfusion and impaired function of multiple organ systems, the limited follow-up data that is currently available provides an optimistic outlook for babies who survive fetal hydrops [33].

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Physiology and Abnormalities of Leukocytes

Kurt R. Schibler

105.1 Physiology of Leukocytes in the Fetus and Neonate

105.1.1 Introduction

The circulating pool of neutrophils reflect a dynamic equilibrium between neutrophil precursors and mature neutrophils in the bone marrow, circulating neutrophils and activated neutrophils that have migrated into the tissues. The bone marrow is the predominant hematopoietic organ after birth. Within the bone marrow, pluripotential hematopoietic stem and progenitor cells give rise to lineage committed neutrophil precursors. These precursors mature under the influence of growth factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF). The bone marrow thus contains pools of immature neutrophils and of stored mature neutrophils. After leaving the bone marrow, neutrophils are transiently in the circulation for a brief period of hours after which they translocate into the tissues.

The peripheral blood neutrophil pool is estimated to contain approximately 5% of the total neutrophil pool. Approximately 1.5 billion neutrophils per kilogram body weight are produced daily. The neutrophil life span consists of about 9 days in the bone marrow, 3–6 hours in the blood, and 1–4 days in the tissues.

Myeloblasts, promyelocytes, and myelocytes are collectively referred to as the neutrophil proliferative pool (NPP) because these cells retain the capacity for cell division. As committed neutrophil precursors mature, their capacity to undergo cell division is lost. This population of postmitotic cells includes metamyelocytes, band neutrophils and mature segmented neutrophils, and is called the neutrophil storage pool (NSP). Neutrophils are released from the NSP to maintain the circulating and marginated neutrophil pools in equilibrium. About 0.3×10^9 neutrophils per kilogram body weight are present in the circulating pool of healthy adults [1].

Adults have a large NSP and can increase circulating neutrophils rapidly in response to infection [2]. By contrast, newborn infants have a relatively small NSP [2]. These differences in the available pool of postmitotic neutrophils limit the ability of newborn infants to respond to the challenges of infections. The circulating neutrophil concentration in humans is relatively low during gestation, but progressively rises until term. Postnatally, the circulating neutrophil count is much higher. Manroe and colleagues described neutrophil ranges in normal term infants during the first 28 days of life [3]. They demonstrated a rise in neutrophil count with a peak between 12 and 24 hours and neutrophil concentrations at that time between 7800 and 14,500 cells per microliter. The neutrophil count subsequently decreased and stabilized after 72 hours with a lower value of 1750 cells per microliter. Mouzihno and coworkers established a reference range for blood neutrophil concentrations in very low birth weight infants [4]. The upper range for normal neutrophil concentration was similar to that of term infants. The lower levels, defining neutropenia, were about 2000 cells per microliter at 12 hours and stabilized at 1000 cells per microliter by 48 hours of life.

The normal circulating monocyte count in the healthy human adult is estimated to be between 300 and 450 monocytes/mm^3. Peripheral blood monocyte counts have been observed to vary in a cyclic fashion over a 3–6 day period [5, 6].

The promonocyte cell cycle rate varies between 30 and 48 hours. These rapidly dividing cells achieve a production rate between 7×10^5 and 7×10^6 monocytes/kg of body weight per hour. After 60 hours of maturation in the bone marrow, mature monocytes move into the intravascular space, where they circulate for approximately 3 days [7]. Van Furth and coworkers [8] calculated that 1.66×10^{10} circulating monocytes leave the intravascular space/hour.

Tissue macrophages are derived from bone marrow precursors. The first evidence for the bone marrow origin of

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

macrophages was obtained in mouse chimeras [9] and rat parabiosis experiments [10, 11]. Studies in patients undergoing bone marrow transplantation, isolation and characterization of lung [12] and liver macrophages [13], provided evidence that these cells contained the karyotype of the bone marrow donor. Thus, terminally differentiated macrophages must have been derived from transplanted bone marrow. Furthermore, by following the disappearance of the Y body–containing macrophages from male patients who have received female donor marrow, the half-life of the alveolar macrophage was estimated to be 81 days.

The size of the tissue macrophage compartment is considerable. According to some estimates, the number of tissue macrophages is 500–1000 times greater than the bone marrow compartment. Some organs, such as the lung and liver, may have particularly large macrophage populations that may account for between 20 and 30% of the total cell number. These cells are long lived and have been estimated to survive for several months [12–14].

105.1.2 Leukocyte Functional Differences Associated with Prematurity

After neutrophils have emigrated from the circulation, cell movement may occur either by random movement or by directed movement toward a chemoattractant. Random movement not related to a stimulus occurs in any direction with equal probability. The ability to undergo directed migration depends upon sensory mechanisms capable of detecting differences in concentration of chemotactic molecules and linking this information to the locomotory apparatus in the cell.

Random movement by neonatal neutrophils is normal compared to that observed in adult cells [15]. However, a number of studies indicate that directed movement of neutrophils from preterm and term neonates to defined chemotactic stimuli is impaired [16–18].

Mononuclear phagocytes exhibit both random and directed movement. Random or nondirected movement occurs in the absence of attracting substances. Directed movement along a concentration gradient of chemical attractants is essential for the effective localization of mononuclear phagocytes to sites of infection and inflammation. This directional movement, called chemotaxis, is governed by chemoattractant molecules that bind to receptors on the cell surface. Chemotactic substances are produced through activation of the complement, fibrinolytic and kinin systems or are directly produced by microorganisms. These chemoattractants may also accentuate random migration of mononuclear phagocytes in the absence of a concentration gradient. An entire family of proinflammatory molecules called chemokines has been identified and many of its members have been characterized [18]. Several members of this family serve as potent chemoattractants for mononuclear phagocytes. Monocyte chemotactic protein 1, macrophage inflammatory protein 1, and RANTES (regulation upon activation, normal T-cell expressed and secreted) are key regulators of monocyte migration. Monocyte-derived dendritic cells migrate to lymphoid organs in response to secondary lymphoid chemokine [19, 20].

Mononuclear phagocyte migration has been examined in cord blood and neonatal peripheral blood. In most reports, random movement by cord blood monocytes appears to be equivalent to that of adult peripheral blood monocytes [21–23]. By contrast, several investigators have reported diminished directed movement by monocytes from cord and neonatal peripheral blood compared with those from adult peripheral blood. Yegin [24] reported that monocyte chemotaxis increased gradually during childhood and was equivalent to adult chemotactic activity at 5–6 years of age. These results are consistent with those of other investigators who reported similar chemotactic activity by cord blood and adult peripheral blood monocytes, but dramatically decreased chemotaxis by neonatal peripheral blood monocytes in the first few days of life [25]. Monocyte chemotaxis gradually increased but remained lower than adult peripheral blood monocyte activity during the first 6 months of life. Other groups of investigators reported slightly increased chemotaxis of cord blood monocytes in response to endotoxin-activated adult serum or activated adult lymphocytes [15, 26]. Differences in methods of measurements applied, patient populations examined and monocyte isolation used, likely account for the contradictory results reported by these investigators.

Neutrophils establish contact with microorgnisms through opsonins, including complement and IgG antibody. The phagocyte completely encircles the microorganisms with circumferential interactions between opsonins and receptors. Once the newly formed vacuole is internalized, the products of intracellular granules are discharged into the vacuole [27, 28]. Mobilization of granules appears to be normal in newborns compared to adults [29]. Neutrophilic granules from newborn infants possess equivalent content of myeloperoxidase and lysozyme, but diminished quantities of lactoferrin and gelatinase [29, 30].

In several experiments, neutrophils from healthy term infants provided with opsonins from adult serum exhibited normal phagocytosis of a variety of microorganisms including *Escherichia coli*, group B Streptococci, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, and *Candida albicans* [31–34]. Phagocytosis by neutrophils from preterm infants, while less well studied, appears to have competent phacocytic functions for bacteria and foreign particles. Ingestion of *Candida albicans* by neutrophils of preterm infants was reported to be normal in one study, but diminished in others [34–36].

The kinetics of phagocytosis by monocytes from cord blood and adult peripheral blood have been studied by Schuit and Powell [37]. These investigators found that monocytes isolated from adult peripheral blood ingested all polystyrene particles within 50 minutes. However, only 38% of cord

blood monocytes had initiated phagocytosis in the same time period. The cord blood monocytes were able to ingest the particles by 100 minutes. These studies were not influenced by the presence of serum or heat-inactivated serum. Other investigators have reported that opsonized (IgG) sheep erythrocytes, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Toxoplasma gondii* and type II herpes simplex are ingested by cord blood monocytes as effectively as monocytes derived from adult peripheral blood [38]. Defective phagocytosis of *Streptococcus agalactiae* by cord blood monocytes has been reported by Marodi and colleagues [39]. Monocytes derived from term cord blood ingested fewer group B streptococci than monocytes isolated from adult blood. Other studies suggested that the phagocytic defect of group B *Streptococcus* by cord blood monocytes could be overcome by inclusion of adhesive glycoprotein fibronectin in the culture. Although fibronectin alone did not enhance phagocytosis, there was enhanced phagocytosis by cord blood monocytes of group B *Streptococcus* preopsonized with IgG preparation in combination with fibronectin.

The phagocytic activity of cord blood monocyte–derived macrophages has been compared with that of macrophages derived from adult blood monocytes. After a 10 day culture period, Speer and co-workers [40] determined that cord blood–derived macrophages ingested complement opsonized *S. aureus* to the same extent as macrophages from adult blood. Other investigations compared the phagocytic activity of alveolar macrophages from intubated newborns with alveolar macrophages from bronchoalveolar lavage of adult subjects. Phagocytosis of *C. albicans* by newborn and adult alveolar macrophages was determined to be equivalent with respect to rate and number of organisms ingested [41].

Table 105.1 Frequency of leukocyte abnormalities

Common
- Bacterial and fungal infection
- Maternal hypertension

Moderately common
- Twin-twin transfusion
- Isoimmune
- Rh hemolytic disease
- Drug induced
- Viral infection (rubella, cytomegalovirus)
- Leukaemoid reaction

Rare
- Severe congenital neutropenia (Kostmann syndrome)
- Cyclic neutropenia
- Shwachman-Diamond syndrome
- Reticular dysgenesis
- Autoimmune neutropenia
- Chediak-Higashi syndrome
- Dyskeratosis congenita
- Cartilage-hair hypoplasia syndrome
- Chronic granulomatous disease
- Leukocyte adhesion deficiency types I and II

105.2 Leukocyte Abnormalities

105.2.1 Etiology and Pathogenesis

The chief disorders of leukocytes observed in the newborn period include quantitative abnormalities such as neutropenia, neutrophilia, and a leukemoid reaction. Qualitative defects in phagocytic leukocytes also occur in conjunction with quantitative abnormalities and with rare hematopoietic disorders. Leukocyte disorders that occur in the neonatal period are categorized based on the frequency of the condition in Table 105.1.

105.2.2 Common Leukocyte Abnormalities

105.2.2.1 Infection

Animal studies comparing the hematopoietic response to infection between neonates and adults have demonstrated differences in the supply and release of neutrophils from the bone marrow [42]. These developmental differences increased the susceptibility of newborn animals to depletion of bone marrow reserves when subjected to experimental infection and ultimately limited their capability to survive such infections [43, 44]. Septic neonates who are neutropenic have a higher mortality than non-neutropenic neonates [45, 46]. Neonates also have an immaturity of neutrophil function and production. Immaturity of granulopoiesis in preterm neonates is manifest by a low neutrophil cell mass, a reduced capacity for increasing progenitor cell proliferation and frequent occurrence of neutropenia in response to sepsis [47]. Neonatal neutrophil function may be reduced in signal transduction, cell surface receptor upregulation, mobility, cytoskeletal rigidity, microfilament contraction, oxygen metabolism and intracellular oxidant mechanisms [48].

105.2.2.2 Associated with Maternal Hypertension

A high proportion of newborn infants delivered to women with hypertension have reduced circulating neutrophil concentrations [3, 4] because of decreased neutrophil production [49], particularly when there is fetal growth restriction. Studies have shown a decrease in neutrophil progenitor cells and decreased cycling of these cells, a relatively normal NSP, and absence of immature neutrophils in the circulation, termed a left shift. Although the precise cause of reduced neutrophil production is uncertain, it does not appear to be related to maternal medications [49, 50]. Non-specific innate host defenses may be impaired and there is a higher incidence of nosocomial infection among infants delivered to hypertensive mothers neutropenic infants compared to non-neutropenic infants [49, 51, 52].

105.2.3 Moderately Common Leukocyte Abnormalities

105.2.3.1 Leukemoid Reaction

Newborn infants may mount an exaggerated response to infection with an incease in neutrophil count and a left shift. There is a significant increase in neutrophil precursors in the peripheral blood as well as an increase in the WBC count exceeding 50,000 cells per microliter is considered a leukemoid reaction. A leukemoid reaction in response to a severe infection in the newborn is frequently accompanied by cytoplasmic vacuoles within the neutrophils and by the appearance of toxic granulations. Infants with Down syndrome may have a type of leukemoid reaction unrelated to infection.

105.2.3.2 Associated with Rh Hemolytic Disease

A well-documented complication of severe Rh erythroblastosis fetalis is a reduction in the concentration of neutrophils and platelets (35, 36). In one series of 20 infants with Rh sensitization, 11 infants had severe disease requiring multiple exchange transfusions (59). Before exchange transfusion all infants with severe disease had neutropenia. The neutrophil kinetic defect persisted between 3 and 5 days in these infants whereas none of the infants with mild disease exhibited neutophil depletion.

105.2.3.3 Isoimmune Neonatal Neutropenia

Isoimmune neonatal neutropenia results from maternal production of IgG directed against antigens on fetal neutrophils (2). This is analogous to Rh hemolytic disease in that maternal sensitization to fetal neutrophil antigens results in transplacentally acquired IgG antibody that destroys the infant's neutrophils (37). Maternal sensitization may occur during gestation and may even affect the fetus of a primagravida (38). The incidence of isoimmune neonatal neutropenia is estimated to be 0.5–2 per 1000 live births (39).

Affected infants frequently develop a fever in the first days of life and are particularly susceptible to cutaneous infections caused by *Stapylococcus aureus*. β-hemolytic *Streptococcus* and *E. coli* have also been linked to infections among susceptible infants with this disorder. The onset of infection is usually concurrent with severe neutropenia. In the circulation, the concentrations of other myeloid lineages particularly monocytes and eosinophils are typically increased. Characteristic findings on bone marrow examination are myeloid hyperplasia with a paucity of mature neutrophils and normal erythropoietic and megakaryocytic elements.

Neutrophil antibodies are detected in the sera of mother and infant. The antibodies react against neutrophils of the patient and of the father, but not against neutrophils from the mother. Several neutrophil-specific antibodies have been implicated including most commonly human neutrophil alloantigens (HNA)-1, HNA-2, and HNA-3. Other antingenic targets include NC1, SH, SAR, LAN, LEA, CN1, and certain HLA antigens. HNA-1 and NC1 have been identified as isotypes of the FcγIII receptor (2). HNA-2 is an antigen on glycoprotein (GP)50 and HNA-3 corresponds to an antigen on GP75-90. The infant's neutrophil counts typically normalize over the first 1–5 weeks of life as might be expected with the half-life of maternal antibodies.

Treatment for affected infants is supportive. Therapy also includes appropriate antibiotic administration for infections and close follow-up. The use of prophylactic antibiotics has been shown to be ineffective. Intravenous immunoglobulin (IVIG) administration and steroid therapy have not been shown to consistently improve circulating neutrophil counts. For infants with persistence of extremely low neutrophil counts (less than 500 cells per microliter), G-CSF administration might be undertaken. This therapy usually results in a prompt clinical response in circulating neutrophil concentrations.

105.2.3.4 Neonatal Autoimmune Neutropenia

Neonatal autoimmune neutropenia results from transplacental passage of maternal IgG autoantibodies directed against neutrophil antigens. Mothers of these infants can be asymptomatic or have autoimmune neutropenia from systemic lupus erythematosis or idiopathic thrombocytopenic purpura (40).

105.2.3.5 Drug-Induced Neutropenia

A significant number of medications have been implicated as causes of the development of neutropenia (Table 105.2). The mechanisms may involve bone marrow suppression or immune-mediated destruction. Marrow recovery generally begins several days after discontinuing the causative agent.

105.2.3.6 Late-Onset Neonatal Neutropenia

Most episodes of neonatal neutropenia occur during the first week of life and are related to low gestational age, low birth weight, infections, maternal hypertension, severe neonatal asphyxia, drug therapy or other perinatal events, or are of unknown cause. By contrast, late-onset neonatal neutropenia, defined as an absolute neutrophil count (ANC) $<1500/mm^3$, is observed in normally growing very-low birth-weight (VLBW) infants at a postnatal age of ≥3 weeks and is not associated to the typical complications that usually precede early-onset neonatal neutropenia (41).

Although less frequent than the early-onset type, late-onset neutropenia may be found in a significant proportion,

Table 105.2 Examples of drugs associated with neutropenia

Anti-inflammatory agents
- Indomethacin
- Pentazocine
- Para-aminophenol derivatives
  - Acetoaminophen
- Pyrozolone derivatives
  - Aminopyrine
  - Dipyrone
  - Oxyphenbutazone
  - Phenylbutazone

Antibiotics
- Cephalosporins
- Clindamycin
- Gentamicin
- Isoniazid
- Penicillins and semisynthetic penicillins
- Rifampin
- Streptomycin
- Trimethoprim-sulfamethoxazole
- Vancomycin

Anticonvulsants
- Carbamazepine
- Phenytoin
- Sodium valproate

Antidepressants
- Amitriptyline
- Amoxapine
- Doxepin
- Imipramine

Antihistamines (H-2 Inhibitors)
- Cimetidine
- Ranitidine

Antimalarials
- Amodiaquine
- Chloroquine
- Dapsone
- Pyrimethamine
- Quinine

Antithyroid
- Carbimazole
- Methimazole
- Propylthiouracil

Cardiovascular
- Captopril
- Dispopyramide
- Hydralizine
- Methyldopa
- Procainamide
- Propranolol
- Quinidine

Diuretics
- Acetazolamide
- Chlorthiadone
- Chlorothiazide
- Ethicrynic acid
- Hydroclorothiazide

Hypoglycemic agents
- Chlorpropamide
- Tolbutamide

Sedatives
- Benzodiezapines
- Meprobamate

Phenothiazines
- Chlorpromazine
- Phenothiazines

up to 22% of VLBW and in 5.5% of low birth-weight (LBW) infants, and is associated with anemia of prematurity and high reticulocyte counts, or the administration of erythropoietin at very high doses (42).

Mean age at onset of neutropenia is about 6 weeks, while the duration may range from 2–7 weeks.

An imbalance between hematopoietic growth factors (mainly erythropoietin and granulocyte colony-stimulating factor) with increased reticulocytopoiesis in response to anemia may explain the neutropenia.

Late onset neutropenia appears to be a benign condition that is not associated with any particular complication and does not require specific treatment. However, investigation should be performed to exclude other causes of neutropenia.

105.2.4 Rare Leukocyte Abnormalities

Hereditary neonatal neutropenia is covered elsewhere (see Chapter 106).

15.2.4.1 Cartilage-Hair Hypoplasia Syndrome

Cartilage-hair hypoplasia is an autosomal recessive disorder characterized by short-limbed dwarfism, fine hair, and moderate neutropenia [53]. Affected individuals have an increased susceptibility to infections, particularly varicella zoster viral infections. Immunologic defects are variable, but cellular immune functions are impaired.

105.2.4.2 Reticular Dysgenesis

Reticular dysgenesis, also called congenital aleukocytosis, is characterized by severe neutropenia associated with leukopenia, presence of rudimentary thymic lymphoid and splenic tissue, and agammaglobulinemia [54]. Histological examination of the bone marrow, spleen and lymphoid tissue reveals normal reticular structure with normal erythroid and megakaryocyte elements, but with absent or sparse myeloid cells. The mechanism of the disorder is unknown, but a defect in lymphohematopoietic progenitor cell development is suspected [55]. Bacterial and viral infections occur early in life and are severe. Aggressive antibiotic therapy and supportive care are necessary for survival. Treatment with G-CSF and GM-CSF have been ineffective. Bone marrow transplantation remains the only long-term treatment option available for infants with this disorder [54, 56].

105.2.4.3 Dyskeratosis Congenita

Dyskeratosis congenita (DC) is an inherited bone marrow failure syndrome clinically characterized by the triad of abnormal nails, reticular skin pigmentation, and oral leukoplakia, and is associated with high risk of developing aplastic anemia, myelodysplastic syndrome, leukemia, and solid tumors. Patients have very short germline telomeres, and approximately half have mutations in one of six genes encoding proteins that maintain telomere function. Accurate diagnosis of DC is critical to ensure proper clinical management, because patients who have DC and bone marrow failure do not respond to immunosuppressive therapy and may have increased morbidity and mortality associated with hematopoietic stem cell transplantation [57].

105.2.4.4 Leukocyte Adhesion Deficiency Type I

Leukocyte adhesion deficiency (LAD) encompasses a rare group of autosomal recessive disorders [58]. LAD type I is associated with defects in neutrophil adhesion and chemotaxis resulting in impaired clearance of complement-opsonized microorganisms. Infants present with delayed separation of the umbilical cord and recurrent infections despite a normal or increased neutrophil count. LAD should be suspected in any

infant with unusually severe bacterial infections accompanied by normal or increased circulating neutrophils and a striking absence of purulent material at the site of infections.

The clinical presentation varies depending on the relative deficiency of CD18. CD18 is the β_2-subunit of the cell surface leukocyte integrin. These molecules are involved in adherence, chemotaxis, C3bi-mediated ingestion, degranulation, and neutrophil respiratory burst. Diagnosis of LAD type I is made by demonstration of a severe deficiency of the β_2-subunit of neutrophil integrins. Carriers can be identified as having about 50% of the normal β_2-subunit levels in circulating neutrophils, whereas affected individuals express 0–10% levels. α/β-Integrins are common on the surface of all neutrophils and make up three distinct cell surface proteins: LFA-1 ($\alpha_1\beta_2$), MAC-1 ($\alpha_m\beta_2$) and P150, 95 ($\alpha_x\beta_2$). Each of these integrins are affected because they require a common β_2-subunit that is diminished in quantity or functionally defective. Several mutations have been identified in the gene coding for the β_2-subunit. These mutations fall into several categories in which there are absent, low or normal level of CD18 mRNA and in which the β_2-subunit protein precursor is absent, low in abundance or abnormal in size. There is a close genotype-phenotype correlation between the molecular defect and the clinical severity of disease.

Leukocyte trafficking from bloodstream to tissue is important for the continuous surveillance for foreign antigens as well as for rapid leukocyte accumulation at sites of inflammatory response or tissue injury. Leukocyte interaction with vascular endothelial cells is a pivotal event in the inflammatory response and is mediated by several families of adhesion molecules. The crucial role of the beta(2)-integrin subfamily in leukocyte emigration was established after LAD type I was discovered. Patients with this disorder suffer from life-threatening bacterial infections, and, in its severe form, death usually occurs in early childhood unless bone marrow transplantation is performed.

The LAD type II disorder (described in more detail below) clarifies the role of the selectin receptors and their fucosylated ligands and patients have a less severe form of infectious episodes (resembling the moderate phenotype of LAD type I) but also severe psychomotor and growth retardation. LAD type III emphasizes the importance of the integrin-activation phase in the adhesion cascade and all hematopoietic integrin activation processes are defective, leading to severe infection as observed in LAD type I and to an increased tendency for bleeding problems due to defective activation of beta(1), beta(2), and beta(3) integrins [58].

105.2.4.5 Leukocyte Adhesion Deficiency Type II

LAD type II is a rare autosomal recessive clinical syndrome involving recurrent bacterial infections, short stature, severe mental retardation, and the hh (Bombay) RBC phenotype. Neutrophils from patients affected with this disorder exhibit markedly diminished chemotaxis *in vitro*, although the neutrophils display normal levels of CD18 and are able to ingest serum-opsonized particles. The molecular defect in these patients is a defect in the fucosyltransferase gene responsible for the carbohydrate linkages associated with the AB blood groups, specifically the sialyl-Lewis X structure. Neutrophil function is defective in these individuals because sialyl-Lewis X is the neutrophil cell surface ligand recognized by endothelial cell surface E-selectin and P-selectin receptors [59].

105.2.4.6 Chediak-Higashi Syndrome

Chediak-Higashi syndrome is a rare, autosomal recessive disorder characterized by the presence of giant cytoplasmic granules in multiple cells throughout the body. The syndrome affects neutrophil and lymphocyte function [60]. Defects in the CHS1 gene family have been implicated in the causation of this disorder. These genes encode proteins involved in vesicular trafficking although the precise function of these proteins is uncertain. Neutrophils display abnormal adherence and chemotaxis, delayed degranulation, and impaired ingestion and killing of microorganisms. Patients with this syndrome are highly susceptible to bacterial infections. Most patients with Chediak-Higashi syndrome exhibit lymphohistiocytic infiltration of multiple organs that appears to result from a lack of natural killer cell function. Death often occurs in the first decade of life from infection, bleeding, or lymphohistiocytic infiltration. Bone marrow transplantation can be effective in treating the hematopoietic complications of Chediak-Higashi syndrome.

105.2.4.7 Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is an inherited immunodeficiency syndrome in which generation of superoxide by the respiratory burst oxidase of phagocytic leukocytes is markedly diminished or absent [61]. The disorder, which has an incidence of approximately one per 500,000 individuals, results from mutations in any one of four essential subunits of the respiratory burst complex. Superoxide generated during the respiratory burst is the precursor for a number of potent oxidants that are important in elimination of many microbial pathogens. Approximately two thirds of CGD cases are caused by defects in the X-linked gene encoding gp91-phox, the large subunit of the flavocytochrome b_{588}, a plasma membrane heterodimer that is the redox center of the phagocyte oxidase [61, 62]. A rare autosomal recessive form of CGD is caused by mutations in the gene encoding the small subunit of the p22-phox flavocytochrome b_{588}. Other cases of autosomal recessive CGD are caused by gene defects in two soluble proteins that interact with flavocytochrome b_{588}, p47-phox or p67-phox.

The clinical manifestations of CGD are apparent during infancy or early childhood [61–64]. Patients with CGD

experience recurrent purulent bacterial and fungal infections that are difficult to treat and often may be life threatening. Common sites of infection are skin, lymph nodes, lungs, bones, liver, and the gastrointestinal tract. Individuals with CGD are particularly susceptible to infections with *S. aureus*, a variety of gram-negative bacilli including *Pseudomonas*, *Salmonella*, and *Serratia* species and fungus such as *Aspergillus* species. Many of these microorganisms express catalase, which prevents phagocytes of CGD patients from scavenging microbe-generated hydrogen peroxide to promote microbial killing within the phagosome. The hallmark of CGD is the propensity to develop chronic inflammatory granulomas with wide spread tissue distribution [63, 65].

The prognosis for individuals with CGD has improved significantly since the disorder was first described in the 1950's. Current management includes aggressive treatment of acute infections in combination with the use of prophylactic antibiotics and interferon-γ (IFN-γ) [66, 67]. Allogenic bone marrow transplantation can be curative even in cases where transplantation results in mixed chimerism [68–70].

Because the genetic defect of CGD primarily affects cells of the hematopoietic system, this disorder has been considered a prime candidate for gene therapy targeted at hematopoietic stem cells [71]. Insertion of a functional copy of the involved gene into hematopoietic stem cells should reconstitute the respiratory burst oxidase activity in circulating and tissue phagocytes and thus could provide a life-long cure of the disease.

105.2.4.8 Autoimmune Neonatal Neutropenia

Autoimmune neutropenia (AIN) is a disorder caused by increased destruction of neutrophils as a result of autoantibodies to the patient's own neutrophils. The incidence is approximately one per 100,000 live births [72]. Primary AIN is not associated with other autoimmune disorders such as systemic lupus erythematosus. While this disorder was definitively identified in 1975, its cause has not been identified [73, 74]. Reports of associations with parvovirus B19 and beta-lactam antibiotics suggest mechanisms such as development of cross-reacting antibodies resulting from molecular mimicry, changes in endogenous antigens, enhanced HLA expression, or loss of suppression of self-reacting lymphocyte clones. HNA-1a (NA-1) autoimmunization has been linked to HLA-DR2, a finding implicating immune response genes [2]. Patients with this immune mediated neutropenia present in the first 3 years of life with a variety of infections. In approximately 80% of patients the infections are mild, consisting of abscesses, conjunctivitis, gastroenteritis, otitis media, pyoderma, and upper respiratory infections. In the remaining cases, AIN predisposes to serious infections such as meningitis, pneumonia, and sepsis [75]. Diagnosis of AIN is made by detection of neutrophil-specific antibodies. Although patients with AIN do not generally require specific treatment for neutropenia, to increase neutrophil counts in cases of severe infection or before elective surgery, some patients have been treated with corticosteroids, IVIG or G-CSF. Corticosteroids and IVIG increased neutrophil counts in about 50% of individuals, whereas G-CSF increased neutrophil counts in all patients treated. Infections are treated symptomatically with antibiotics. Infants with recurrent infections are often treated with prophylactic antibiotics.

105.2.5 Clinical Aspects

Classification of leukocyte abnormalities according to the underlying kinetic mechanism can provide useful clues for diagnosis and management of affected infants. The complete blood count and differential white count provides an estimate of the responsible kinetic mechanism by determining the degree of left shift, or the ratio of immature to total neutrophils in the blood. Neutropenia accompanied by a large left shift is likely to be on the kinetic basis of accelerated neutrophil destruction or usage [44, 76]. A large left shift is defined as a ratio of immature (band neutrophils or metamyelocytes) to total neutrophils of greater than 0.3. By contrast, neutropenias in which only mature neutrophils are observed in the blood are more likely to be on the kinetic basis of diminished neutrophil production or excessive margination [49, 77–79].

The variety of leukocyte abnormality is often suggested by associated findings (Table 105.3). For instance, neutropenia in an infant with intrauterine growth restriction delivered to a mother with hypertension, is likely to have neutropenia based on diminished neutrophil production, as commonly seen in that condition [49, 78]. The presence of neutropenia in the mother of an infant with neutropenia should prompt evaluation for maternal autoimmune antibodies causing immune-mediated neutrophil destruction. The presence of steatorrhea in a neutropenic neonate should prompt consideration of Shwachman-Diamond syndrome [80–82]. Severe and prolonged neutropenia, with counts typically less than 200 cells per microliter associated with monocytosis and eosinophilia

Table 105.3 Associated findings in newborn patients with leukocyte abnormalities that can suggest a specific disorder

Associated Finding	Variety of Leukocyte Abnormality
Albinism	Chediak-Higashi syndrome
Anemia and multiple gestation	Donor in twin-twin transfusion
Blueberry muffin rash	Congenital cytomegalovirus
Down syndrome	Leukemoid reaction
Hydrops	Rh hemolytic disease
Monocytosis and eosinophilia	Kostmann syndrome
Mother with hypertension	Neutropenia of PIH
Mother with neutropenia	Maternal autoimmune neutropenia
Pancytopenia	Reticular dysgenesis
Shock	Endotoxemia or sepsis
Steatorrhea	Shwachman-Diamond syndrome

on peripheral blood smear is seen in Kostmann syndrome where bone marrow examination is characterized by arrested neutrophil development at the promyelocyte or myelocyte stage [83, 84]. Neutrophilia with immature circulating myeloid elements in a stable infant with Down syndrome should prompt long-term follow-up because, although the neutrophilia is generally transient, up to 30% of these infants may develop leukemia later in childhood [85]. Neutrophilia or neutropenia with a left shift in conjunction with clinical signs of shock will prompt early and aggressive antibiotic treatment and supportive care in addition to investigation to identify the causative microbial agent. An association of neutrophilia and the presence of a "blueberry muffin rash", which is often indicative of extramedullary hematopoiesis, might prompt evaluation for congenital viral infections such as cytomegalovirus (CMV).

105.2.6 Differential Diagnosis

When evaluating a neonate with leukocyte abnormalities it can be helpful to maintain the perspective that some varieties of these abnormalities are common and others are exceedingly rare. The most common causes of leukocyte abnormalities among infants have an obvious underlying cause. One form of neutropenia, where there is a large left shift or an immature to total neutrophil ratio exceeding 0.3–0.5, accompanies overwhelming sepsis. However, another form of neutropenia (generally with no left shift or an immature to total neutrophil ratio of less than 0.2) occurs in infants with intrauterine growth restriction born to a woman with pregnancy-induced hypertension. In general these common varieties of neutropenia do not require additional investigations. Neutropenia accompanying sepsis resolves quickly if the patient survives. However, if neutropenia in the infant with sepsis persists for several days, additional investigations should be considered. Similarly, in most cases of neutropenia accompanying pregnancy-induced hypertension, neutropenia resolves within 5 days. If neutropenia persists for more than 5 days additional investigations may be considered, particularly if the neutrophil count is less than 500 cells per microliter.

Laboratory tests to be considered are those that provide a specific diagnosis, such as alloimmune or autoimmune neutropenia. These tests, while not diagnostic, are required to investigate rare forms of neutropenia such as congenital and cyclic neutropenia. Obtaining a complete blood count on the infant, including microscopic examination of neutrophil morphology, can be useful in identifying features such as left shift, monoytosis, eosinophilia, and the presence of cytoplasmic inclusions formed by fused lysosomes. Monocytosis and eosinophilia are commonly observed in patients with congenital neutropenia and cytoplasmic inclusions and are characteristic of Chediak-Higashi syndrome [60]. Also, a complete blood count on the mother to establish her neutrophil concentration can be useful to detect cases of autoimmune neutropenia in which mother has immune-mediated neutropenia, which is passively acquired by the fetus. Maternal and infant neutrophil antigen typing and anti-neutrophil antibody determination should identify most cases of immune-mediated neutropenia.

To evaluate neonatal immune mediated neutropenia, antibody testing is performed on both maternal and infant blood samples including two screening tests: 1) a neutrophil agglutination assay and 2) a granulocyte immunofluorescence assay. If either assay is positive, an HLA screen is performed because HLA antibodies can react with neutrophil assays and it cannot be determined whether the antibody is neutrophil specific or HLA related. When this occurs, an additional test with antigen capture assay is used. In this assay, monoclonal antibody immobilization of neutrophil antigens is done to differentiate between neutrophil antigen-specific antibodies from HLA antibodies [86, 87]. This assay can detect more than one neutrophil antigen-specific antibody.

A bone marrow study may be useful for patients with severe and prolonged neutropenia in whom isoimmune and maternal autoimmune neutropenia have been excluded. Bone marrow aspirate or biopsy may suggest the kinetic mechanism underlying the neutropenia by estimation of proliferative myeloid cells compared to postmitotic myeloid cells. For example, when neutropenia is caused by diminished neutrophil production, the proliferative compartment is decreased. In accelerated neutrophil usage or destruction, the proliferative compartment responds by expanding production. In addition, mature neutrophils are rapidly released into the peripheral blood, simulating a maturational arrest or decreased postmitotic pool. Despite its usefulness in narrowing the differential diagnosis, bone marrow studies almost never provide a precise diagnosis. For instance, no pathognomonic feature of bone marrow biopsy discriminates completely between hyporegenerative neutropenias in the neonatal period such as between neutropenia associated with maternal hypertension or Shwachman-Diamond syndrome. In a few instances, there are useful morphological clues in the bone marrow. For example, congenital neutropenia syndrome is suggested by enlargement and binucleation of promyelocytes and other myeloid precursors [84]. In autoimmune neutropenia, the bone marrow may contain macrophages with ingested antibody-coated neutrophils.

Neutrophil counts vary considerably early in the neonatal period with a mean of about 11,000 cells per microliter and a range between 6000 and 26,000 cells per microliter [3]. After the first 12 hours of life, neutrophil counts fall reaching a mean of 5000 cells per microliter (ranging from 1000 to 10,000 cells per microliter). Neutrophilia, defined as an elevation of the circulating absolute neutrophil count more than two standard deviations above the mean, may occur in response to both bacterial and viral infections, stress (post-operative or after seizures), or in conjunction with hemolytic anemia or immune thrombocytopenia. Occasionally, newborn infants exhibit an exaggerated response to infection with a significant increase in total WBC count (50,000 cells per

microliter) and increased early myeloid precursors in the peripheral blood, termed a leukemoid reaction [88]. The leukemoid reaction observed with a severe infection is often accompanied by cytoplasmic vacuoles within neutrophils and by the appearance of toxic granulations. The duration of the reaction ranges from days to weeks. Approximately 10% of infants with Down syndrome may have a transient leukemoid reaction that resembles congenital leukemia [85]. It is characterized by the presence of megakaryoblasts in the peripheral blood, hepatosplenomegaly, variable thrombocytopenia and infrequently hydrops fetalis and severe hepatic fibrosis. Abnormalities in all three hematopoietic lineages have been described. In most cases the leukemoid reaction is transient, although up to 30% of infants with Down syndrome infants who experience a neonatal leukemoid reaction develop acute megaloblastic leukemia later in childhood [85]. Leukaemoid reactions have also been reported in phenotypically normal infants who have trisomy 21 mosaicism associated with a clonal trisomy 21 in bone marrow cells [89, 90].

105.2.7 Prognosis

105.2.7.1 Infection

Infection remains a major cause of illness and death in the neonatal period [91, 92]. Newborn babies have an immature immune system and therefore may not show all signs of infection, and delayed treatment may lead to severe illness or death [93, 94]. Early treatment with antibiotics has been shown to reduce mortality due to sepsis in the neonatal period. Early treatment depends on a knowledge of risk factors and picking up early signs of infection [93, 94], which tend to be non-specific in this age group [95].

Although advances in neonatal intensive care have led to improved survival of very low birth weight (VLBW) and extremely premature infants, late onset sepsis (systemic infection after 48 hours of age) continues to be a significant cause of morbidity and mortality. The incidence of late onset sepsis increases with both decreasing birthweight and gestational age, and has been reported as occurring in approximately 25% of VLBW infants [96, 97]. Infants with the lowest birth weights are also more likely to have multiple episodes of sepsis [96]. In developing countries infection is estimated to cause 30–40% of neonatal deaths [98]. The spectrum of organisms responsible for early onset (vertically transmitted) sepsis differs from that associated with late onset (nosocomial) sepsis. This pattern becomes apparent from day two onwards [99]. Nosocomial infections are frequently associated with clinical deterioration including increased frequency of apnea or ventilatory requirements, temperature instability, abdominal distension, acidosis, lethargy, septic shock, necrotizing enterocolitis, meningitis and death [100]. The complications of necrotising enterocolitis and meningitis predispose an infant to an increased risk of future neurological impairment [101–103] and the mortality from late onset sepsis remains high, at 7–10% [96, 99]. This risk is secondary to immature immune responses, poorly developed skin and mucosal barriers to infection, numerous entry portals for organisms via cannulae, catheters and endotracheal tubes and continuing exposure to opportunistic organisms during a hospital stay, which is often prolonged.

105.2.7.2 Associated Abnormalities and Malignant Transformation

Congenital bone marrow disorders manifest by leukocyte defects are often associated with other congenital abnormalities. For instance, infants with Down syndrome may present with a variety of abnormalities in non-hematopoietic organ systems including cardiac and gastrointestinal malformations. Infants with Shwachman-Diamond syndrome exhibit exocrine pancreatic disorders and short stature. Barth syndrome is a mitochondrial disorder with associated development of cardiomyopathy and acidoisis. A predisposition to hematologic malignancy, in particular acute myelogenous leukemia (AML), is common among several of these disorders. It is postulated that the genetic disorder underlying these conditions provides a first hit expressed as variety of physical abnormalities, and a second hit later in life causes malignant transformation [104]. Downs syndrome, dyskeratosis congenita, Kostmann syndrome, and Shwachman-Diamond syndrome are among the syndromes associated with malignant transformation. Chronic granulomatous disease and cyclic neutropenia are not associated with malignant transformation.

105.2.8 Therapy and Treatment

105.2.8.1 Antibiotics

Early neonatal sepsis is mainly acquired from the mother. Vertical transmission of infection from mother to infant may take place before birth, during labor, or at the time of delivery. Most infants with peripartum acquired sepsis will develop clinical symptoms of sepsis within two days of life. After this period, nosocomial and community acquired infections start to play a bigger role.

The bacteria most commonly implicated in early neonatal sepsis are Group B *Streptococcus* and Gram-negative bacilli, and usually exclude coagulase negative *Staphylococcus*. Neonatal intensive care units or special care baby units tend to choose empirical first line antibiotic therapy that will cover both Gram-negative and Gram-positive bacteria. A combination of an aminoglycoside such as gentamicin and a beta-lactam such as penicillin has been the treatment of choice for early neonatal sepsis.

The range of organisms causing late onset sepsis includes gram positive and gram negative bacteria as well as fungal infection. As bacterial infections predominate, empiric antibiotic regimens focus on cover for both gram positive and negative bacterial infection. These antibiotics can be either narrow or broad spectrum in the range of organisms that they target. The epidemiology of late onset infection differs between developing and developed countries in the incidence of infection, the organisms responsible, and the subsequent mortality rates.

In general, prophylactic antibiotics are recommended for children with chemotherapy-induced neutropenia and children with chronic neutropenic conditions whose neutrophil count is less than 500 cells per microliter until recombinant G-CSF administration raises the neutrophil count to greater than 1000 cells per microliter [105, 106]. Broad use of prophylactic antibiotics for neutropenic neonates is not recommended because in most cases neutropenia is of limited duration and the general use of antibiotics may contribute to the emergence of bacteria with high antibiotic resistance. Individualization of care to include prophylactic antibiotics might be considered for an infant with severe neutropenia (500 cells per microliter) continuing for several days and requiring treatment with G-CSF to increase the neutrophil count above 1000 cells per microliter. Prophylactic antibiotics might also be considered for an infant with severe prolonged neutropenia who is unresponsive to G-CSF treatment. The precise antibiotics used in such cases should be selected based on the local nursery bacterial epidemiology.

105.2.8.2 Granulocyte Transfusions

The availability of new generations of effective antibiotics, recombinant hematopoietic growth factors to counter neutropenia, the risk of transmission of infection and the necessity for appropriate technology are some of the factors that have diminished the enthusiasm for use of granulocyte transfusions in neonates [107]. While there has been a resurgence of interest in enthusiasm for granulocyte transfusions in neutropenic patients, especially in patients with cancer, no recent studies have been conducted in newborn infants [108–112].

A recent systematic review evaluated the role of granulocyte transfusions as an adjunct to antibiotics in the treatment of neutropenic septic newborns [113]. This review included four eligible trials conducted on a small number of randomized infants [114–117].

There was no significant difference in mortality due to any cause during hospitalization in infants with sepsis and neutropenia who received granulocyte transfusions when compared with placebo or no granulocyte transfusion. In a single study by Cairo and coworkers, a reduction in all-cause mortality during hospital stay of borderline statistical significance, was observed when granulocyte transfusions were compared to intravenous immunoglobulin [117].

Granulocyte concentrates used for transfusion have potential adverse effects, including pulmonary complications, transmission of infections, fluid overload and graft versus host disease. Preparation of granulocytes for transfusion requires technical expertise and this is not universally available. Even among centers where it is available, there may be a delay in the procurement and transfusion of granulocytes after a decision to transfuse has been made. This delay may potentially render these transfusions of granulocytes less effective for neonates with neutropenia and sepsis.

105.2.8.3 IVIG

IVIG for Preventing Infection in Preterm and/or Low Birth Weight Infants

Administration of intravenous immunoglobulin provides IgG that can bind to cell surface receptors, provide opsonic activity, activate complement, and promote antibody dependent cytotoxicity. Intravenous immunoglobulin thus has the potential of preventing or altering the course of nosocomial infections. Ohlsson and Lacy conducted a systematic review to assess the effectiveness and safety of intravenous immunoglobulin (IVIG) administration compared to placebo or no intervention to preterm and/or low birth weight (LBW, less than 2500 gram birth weight) infants in preventing nosocomial infections [118]. Nineteen studies were included in this review, the most recent trial being in 2000. These included approximately 5000 preterm and/or LBW infants. Among qualifying studies the quantity of IVIG per dose varied widely from 120 mg/kg [119] to 1 g/kg [120]. Also, the number of doses varied from a single dose [119, 121–124] to seven doses [125]. Several different IVIG preparations were used. When all studies were combined the meta-analysis indicated that IVIG administration resulted in a 3% reduction in sepsis and a 4% reduction in any serious infection, one or more episodes, but was not associated with reductions in other important outcomes: sepsis, necrotizing enterocolitis, intraventricular hemorrhage, or length of hospital stay. Most importantly, IVIG administration did not have any significant effect on mortality from any cause or from infections. Prophylactic use of IVIG was not associated with any short-term serious side effects. From a clinical perspective the small reduction in nosocomial infections without a reduction in mortality or other important clinical outcomes is felt to be of marginal importance.

Antistaphylococcal Immunoglobulins to Prevent Staphylococcal Infection in Very Low Birth Weight Infants

Nosocomial infection continues to be a major problem affecting the immediate health and long-term outcome of preterm and very low birth weight neonates. More than half of these infections are caused by staphylococci. Various type specific

antibodies targeted at different antigenic markers of *Staphylococcus* have been developed and have shown promise in animal studies.

Shah and Kaufman conducted a systematic review to evaluate the efficacy and safety of anti-staphylococcal immunoglobulins in the prevention of Staphylococcal infection in very low birth weight infants [126]. Three eligible studies were included testing two different anti-staphylococcal immunoglobulin products. Two studies [127, 128] used pooled generic anti-staphylococcal immunoglobulin (INH-A21) and the third study [129] used antibody against type 5 and type 8 capsular polysaccharide antigen (Altastaph). These studies enrolled a total of 2,701 neonates. No significant differences were noted in the risk of staphylococcal infection or the risk of any infection between INH-A21 and placebo or Altastaph versus placebo. Furthermore, no significant differences were observed in the incidence of chronic lung disease, patent ductus arteriosus, necrotizing enterocolitis, intraventricular hemorrhage, retinopathy of prematurity or duration of antibiotic and vancomycin use. At the present time, anti-staphylococcal immunoglobulins (INH A-21 and Altastaph) are not recommended for prevention of staphylococcal infections in preterm or VLBW neonates. Further research to investigate the efficacy of other anti-staphylococcal products such as Pagibaximab under development may be forthcoming in the future.

IVIG for Treatment of Infections in Neonates

Ohlsson and Lacy conducted a systematic review to assess the effectiveness of intravenous immunoglobulin (IVIG) in reducing mortality and morbidity caused by suspected and proven infection in newborn infants [130]. Five hundred fifty three neonates with suspected infection were enrolled in randomized clinical trials in seven countries to evaluate the effect of IVIG on neonatal outcomes [131–138]. Six studies enrolling 318 infants reported on the outcome of mortality for randomized patients with clinically suspected infection. Results of the meta-analysis showed a reduction in mortality following IVIG treatment of borderline statistical significance. Treatment with IVIG (seven trials, n = 262) in cases of subsequently proven infection resulted in statistically significant reduction in mortality. There is insufficient evidence to support the routine administration of IVIG preparations investigated to prevent mortality in infants with suspected or subsequently proved neonatal infection. Moreover, well-designed trials will be required to confirm or refute the effectiveness of IVIG to reduce adverse outcomes in neonates with suspected infection.

105.2.8.4 G-CSF and GM-CSF

In the United States, G-CSF is approved for use by the Food and Drug Administration for use in patients with severe chronic neutropenia, cancer patients receiving myelo-suppresive chemotherapy, cancer patients receiving bone marrow transplantation and patients undergoing peripheral blood hematopoietic stem cell collection. Patients with severe chronic neutropenia generally derive considerable benefit from G-CSF administration. Varieties of neutropenia in neonates for which G-CSF treatment is effective are Kostmann syndrome, Shwachman-Diamond syndrome, cyclic neutropenia, and alloimmune neutropenia.

105.2.8.5 Bone Marrow and Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation remains the only available treatment for patients with congenital neutropenia refractory to G-CSF treatment. Absence of response to G-CSF, G-CSF receptor mutation, and leukemic transformation are absolute indications for hematopoietic stem cell transplantation if a suitable donor is available. Pulmonary mycosis and pulmonary abscesses do not represent absolute contraindications to bone marrow transplantation, although a relapse rate of 30–50% for mycosis has been reported, despite adequate medical and surgical treatment [139, 140].

G-CSF has had a major impact on the management of severe congenital neutropenia, cyclic neutropenia, and Shwachman-Diamond syndrome. Almost all patients respond to G-CSF with increased neutrophils, reduced infections, and improved survival. Some responders with congenital neutropenia and Shwachman-Diamond syndrome have developed myelodysplastic syndrome and acute myeloid leukemia, which raises the question of the role of G-CSF in pathogenesis. The issue is complicated because both disorders have a propensity for myelodysplastic syndrome or acute myeloid leukemia as part of their natural history. To address this, the Severe Chronic Neutropenia International Registry used its large database of chronic neutropenia patients treated with G-CSF to determine the incidence of malignant myeloid transformation in the two disorders, and its relationship to treatment and to other patient characteristics. No statistically significant relationships were found between age at onset of myelodysplastic syndrome or acute myeloid leukaemia and patient gender, G-CSF dose, or duration of G-CSF therapy. What was observed, however, was the multistep acquisition of aberrant cellular genetic changes in marrow cells from patients who transformed, including activating ras oncogene mutations, clonal cytogenetic abnormalities, and G-CSF receptor mutations.

In murine models, G-CSF receptor mutation produces a hyperproliferative response to G-CSF, confers resistance to apoptosis, and enhances cell survival. Since congenital neutropenia and Shwachman-Diamond syndrome are inherited forms of bone marrow failure, G-CSF may accelerate the propensity for myelodysplastic syndrome and acute myeloid leukaemia in the genetically altered stem and progenitor cells,

especially in those with G-CSF receptor and ras mutations (82% and 50% of patients who transform, respectively). Alternatively, and equally plausible, G-CSF may simply correct neutropenia, prolong patient survival, and thus allows time for the malignant predisposition to declare itself. In patients who transform to overt myelodysplastic syndrome or acute myeloid leukemia, hematopoietic stem cell transplantation is the only chance for cure. In those with an isolated clonal cytogenetic change, but without other evidence of myelodysplastic syndrome, or with an isolated G-CSF receptor mutation, there is room for conservative management. One option is to reduce the G-CSF dosage as much as possible, and observe progression, if any, to more overt signs of malignancy [141].

Reticular dysgenesis is a very rare congenital immunodeficiency classified within the severe combined immunodeficiencies and characterized by impairment of both lymphoid and myeloid cell development. Bertrand and colleagues reported 10 patients with reticular dysgenesis, treated with HLA-haploidentical hematopoietic stem cell transplantation. All children but one were symptomatic within the first days of their lives. Five patients required two hematopoietic stem cell transplants. Five patients received conditioning therapy with busulfan and cyclophosphamide. Three of them survived with myeloid and T- and B-cell lymphoid reconstitution, whereas two patients died (one chronic graft-versus-host disease, one pneumonitis). Transplantation without or with other conditioning regimens in the other five cases led to absent or incomplete engraftment and none of these cases survived. These results demonstrated the importance of intensive conditioning before haploidentical hematopoietic stem cell transplantation in reticular dysgenesis to achieve full lymphoid and myeloid engraftment [54, 56].

Curently allogenic hemopoietic stem cell transplant is the only curative treatment available for severe congenital neutropenia, leukocyte adhesion deficiency, and chronic granulomatous disease [142].

105.2.8.6 Gene Therapy

Chronic granulomatous disease is a rare congenital disorder resulting from a failure of neutrophils to produce oxidases. Patients are therefore prone to recurrent infections from various organisms including fungi and atypical bacteria. The mortality in patients with the X-linked form of chronic granulomatous disease, the most common type, ranges from 3% to 5% per year and although management of infections has improved with advances in antimicrobial therapies, better methods are needed to cure these patients. Peripheral blood stem cell or bone marrow transplantation, while curative, is not widely used due to the episodic nature of the infections and the belief by many that conservative management is preferable to the risks of transplantation [143]. Still, as will be discussed, improvements in the field are making allogenic transplantation more desirable and tilting the risk benefit ratio in favor of this modality. Additionally, gene therapy, which has been considered a possible method to cure chronic granulomatous disease, has within the last 5–10 years become more and more of a reality and may be realized in the not to distant future [144, 145].

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Neonatal Hereditary Neutropenia

Gaetano Chirico and Carmelita D'Ippolito

106.1 Introduction

Hereditary neutropenia includes many disorders of distinct origin and variable prognosis, characterized by a reduction of the absolute neutrophil count (ANC) that predisposes patients to bacterial infections, in particular pyogenic infections, such as cutaneous cellulitis, deep abscesses, pneumonia and sepsis [1, 2]. Susceptibility to bacterial infections, even in patients with severe neutropenia, can be quite variable, depending on the underlying etiology. Congenital neutropenia my be associated with extraematopoietic manifestations.

106.2 Severe Congenital Neutropenia (SCN)

In 1956 Kostmann described congenital neutropenia as an autosomal recessive disease in a large intermarried Swedish family. The incidence is estimated to be approximately 2 cases per million of the population [1]. Affected patients regularly have episodes of fever, skin infections, stomatitis, pneumonia and perirectal abscesses that usually begin in the first months of life and lead to death during infancy and childhood. The ANC is less than 0.2×10^9 cells/L. Eosinophylia, monocytosis and splenomegaly may be present.

The bone marrow examination reveals the typical arrest of the myeloid cell differentiation at the promyelocytic stage with a marked depletion of mature neutrophils [1, 3].

Recent studies on the genetic basis of SCN have detected mutation in the leucocyte elastase gene, *ELA2*, a gene defect that is inherited as an autosomal dominant trait in about 60–80% of patients [4], a gene defect that is inherited as an autosomal dominant trait. *ELA2* encodes a serine protease synthesized during the promyelocyte/myelocyte stage, which is stored in the primary granules [4]. Correct localization of the neutrophil elastase (NE) in the primary granules requires interaction of NE with the adaptor protein complex AP3 that shuttles transmembrane cargo proteins from the Golgi network to the lysosomes. Most mutations in *ELA2* remove the tyrosine-based recognition sequence for the AP3μ subunit, thereby favoring the misallocation of the enzyme to the membrane instead of inside the granules. It was proposed that mislocalized NE must drastically reduce the production of neutrophils from the myeloid progenitor cells in favor of monocytes.

The introduction of hematopoietic growth factors has greatly improved both life span and quality of life [5]. However, patients with SCN have a heterogeneous response to G-CSF therapy and often require a gradual increase of the dosage that generally ranges from 11 to 13 μg/kg/die. In addition 3–5% of SCN patients are refractory to G-CSF treatment.

Finally a significant number of children who are receiving G-CSF therapy are developing myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML), the overall risk being between 9 and 13% [3]. The exact relationship between G-CSF treatment and the risk of leukemic transformation is still unclear [5]. A subset of SCN patients, namely those with more severe disease, are more prone to develop MDS, AML or both, probably because of exposure to other leukemogenic factors such as monosomy of chromosome 7, alterations of chromosome 21, activating mutations of the oncogene *ras*, and mutation in the G-CSF receptor (*CSF3R*) [5].

In the light of this finding G-CSF treatment seams a safe option for the vast majority of patients with neutropenia, while patients at risk for leukemic transformation would benefit from hematopoietic stem cell transplantation.

106.3 Cyclic Neutropenia

Cyclic neutropenia is an autosomal dominant or sporadic condition, characterized by periodic episodes of severe neutropenia with a nadir of less than 0.2×10^9 cells/L, that occur usually every 21 days [6].

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

The neutropenic periods persist for 3–5 days and are accompanied by malaise, fever, gingivitis, oral ulcers and lymphoadenopathy. There are also regular oscillations of the other leukocyte subsets, reticulocytes and platelets. Symptoms begin during the first year of life, are often milder after puberty and are usually less severe than in SCN [3, 4, 6] even though fatal *Clostridium* bacteremia has been reported in untreated patients [3]. A variety of studies suggest that cyclic neutropenia occurs because of a periodic disruption in cell production in the bone marrow [3]. And it has recently been shown that a heterozygous mutation of *ELA2* is the genetic cause of the disease. The mutations reported in cyclic neutropenia are located in the same gene associated with SCN, but in different exons resulting in preferential accumulation of NE in granules – an example of genotype/phenotype correlation.

In the management of those patients the use of prophylactic G-CSF treatment is recommended, it has been very effective in improving peripheral blood neutrophil counts and avoids symptoms and infections [5]. The dose of G-CSF required is usually lower than in SNC: 2–3 μg/kg/die or on alternate days, the occurrence of MDS or leukemia has not been reported in patients with cyclic neutropenia [5].

106.4 Myelocathexis and WHIM Syndrome

Myelocathexis is a rare autosomal dominant disorder characterized by moderate to severe chronic neutropenia resulting from the retention of mature neutrophils in the bone marrow.

Myelocathexis is often associated with hypogammaglobulinemia, leucopenia and warts, a clinical picture recognized as WHIM syndrome (warts, hypogammaglobulinemia, infections, myelokathexis) [7], an autosomal dominant disease.

In WHIM syndrome ANCs are usually $<0.5\times10^9$/L, but during infectious episodes their number rises suddenly, allowing a more benign course with recurrent mild respiratory infections. Early death related to infections has not been reported. Recent studies showed that WHIM syndrome is caused by heterozygous mutations in the gene coding for the chemokine receptor CXCR4. Cells expressing the mutated CXCR4 have an increased responsiveness to chemokines and thus are not released from the bone marrow to the circulating blood pool [7]. The neutropenia associated with myelocathexis and WHIM is only partially corrected by administration of G-CSF [8].

106.5 Congenital Neutropenia with Extra Hematopoietic Manifestations

106.5.1 Hermansky-Pudlak Syndrome 2

This autosomal recessive disease is characterized by neutropenia, oculocutaneous albinism and moderate bleeding disorders [9]. It is caused by mutations of the gene encoding for the beta3 component of the AP3 complex, again preventing the transport of NE (and other proteins) from the Golgi network to the lysosomes in hematopoietic cells and in melanocytes [9].

The severe neutropenia is responsive to G-CSF treatment.

106.5.2 Shwachman-Diamond Syndrome

The Shwachman-Diamond syndrome (SDS) is a rare multiorgan disease inherited as an autosomal recessive trait that combines neutropenia, exocrine pancreatic insufficiency, skeletal abnormalities and short stature [10].

The symptoms begin early in infancy with bacterical infections (pneumonia, otitis media, osteomyelitis, skin infections, sepsis) and failure to thrive because of intestinal malabsorption.

Chronic neutropenia is constantly observed in all patients and two thirds of them have a neutrophil count less than 1×10^9 cells/L, so while the neutropenia can be intermittent it is never cyclic. Mild anemia and thrombocytopenia are described commonly [10].

Cytopenia reflects hematopoietic dysplasia, which, in association with cytogenetic abnormalities, may increase the risk of transformation in MDS/AML mainly in older children. For this reason annual bone marrow aspirates are recommended in patients with SDS. Indeed follow-up studies of SDS patients demonstrate that some cytogenetic changes my spontaneously regress.

The causative gene of the disease has recently been identified and was named *SDBS*. It is expressed in both hematopoietic and non hematopoietic tissues. Treatment of patients suffering from SDS includes pancreatic enzyme replacement and administration of G-CSF, which increases ANC to normal levels and should be started in case of severe infections [11].

106.5.3 Neutropenia Associated with Glucose-6-Phosphatase Complex Disorders

Glucose 6 phosphatase is a complex of three proteins bound to the endoplasmic reticulum responsible for glycogenolysis. Two of these three proteins are associated with congenital neutropenia: the translocase (SLC37A4), and G6PC3 that is a catalytic protein.

The association between these molecular changes and neutropenia is not clear because the glycogenolysis pathway is not the source of energy normally used by neutrophils, which mainly use the pentose pathway; this raises the hypothesis that this protein has another functions in neutrophils.

106.5.3.1 Glycogen Storage Disease Type Ib

It is a metabolic disorder characterized hepatic glycogen accumulation, intolerance of fasting, hypoglycemic events, and hyperlactacidemia, as well as susceptibility to infections [12] and colitis resembling Crohn's.

This susceptibility to infections is due to neutropenia and, sometimes, to neutrophil dysfunction (defective chemotactism). The origin of the neutropenia and neutrophil dysfunction is unknown.

106.5.3.2 G6PC3 Mutations

G6PC3 mutation prevailing in Armenia is caracterized by severe permanent neutropenia with granulocyte maturation arrest, susceptibility to infections, and several other clinical manifestations, (thin skin, urogenital malformations, and cardiac disorders). Mutations of the G6PC3 gene are generally homozygous, but a double heterozygote has been described [13].

106.5.4 Neutropenia Associated with Poikilodermia, Clericuzio Type

Clericuzio type neutropenia with genodermatosis that onset in the first year of life. Recurrent infections occur, and especially pneumonia. The neutropenia is often severe. The poikilodermia includes skin atrophy and popular erythematous rash. Composite mutations of the C16ORF57 gene are responsible of pathology [14].

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Therapy with Recombinant Leukocyte Growth Factors

Robert D. Christensen

107.1 Discovering Neutropenia in a Neonate

The complete blood count (CBC) is one of the most commonly obtained laboratory tests in Neonatology. When a neonatologist finds a low blood concentration of neutrophils, it can be expected as in a neonate with septic shock, or it can be unanticipated and puzzling. A neonatologist finding neutropenia might wonder: What conditions should I consider in the differential diagnosis? Will the neutropenia be a significant clinical problem or will it be trivial and transient? Should I order rG-CSF? This chapter will focus on the last of these questions, reviewing the studies and the reasoning involved in this decision, and will include an algorithm as a guide to answering this question.

Preparatory to that discussion, it is imperative to first define neutropenia. Consider the number of neutrophils per microliter of blood, also known as the ANC (absolute neutrophil count) and not just the WBC (white blood cells or leukocytes per microliter). If the ANC is less than 1000/μL the neonate has neutropenia. If the ANC is < less than < 500/μL the patient can be said to have severe neutropenia [1–3]. Counts above 1000/μL can technically be neutropenic, if they fall below the 5th% reference range.

For instance, as shown in Fig. 107.1 an ANC of 3000/μL 12 hours after birth is indeed an abnormally low ANC [4, 5]. The fact that the ANC is below the reference range signals the presence of pathology. However, it is doubtful that an ANC as high as 3000/μL constitutes a host-defense deficiency, or renders the patient at high risk for acquiring an infection. Therefore we recommend using the diagnosis neutropenia, or placing neutropenia on the problem list, only if the ANC is less than 1000/μL.

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107.2 The Importance of Neutrophils in Neonatal Host Defense

Neutrophils are pivotal to the process of antibacterial defense [6, 7], and patients lacking neutrophils can experience repeated local and systemic infections [8, 9]. Severe chronic neutropenia (SCN) is a cluster of diagnoses bearing the common feature of neutropenia, generally severe neutropenia, present from birth [10, 11].

The advent of recombinant granulocyte colony-stimulating factor (rG-CSF) dramatically improved the lives of patients with SCN, elevating their circulating neutrophil concentrations, markedly reducing infectious illnesses, and extending their life expectancy [8, 12].

SCN can be diagnosed in a neonate [13, 14]. However, the majority of patients with SCN are not diagnosed until they are several months old, following many infectious episodes that prompted evaluations into immunological deficiencies. When SCN is diagnosed in a neonate, that patient should receive the benefit of rG-CSF treatment [8, 12–15]. When a transient variety of neonatal neutropenia is diagnosed, distinct from SCN, the benefit of rG-CSF treatment is speculative and unproven [16–20]. This chapter will review the biological plausibility, the animal studies, and the clinical trials aimed at testing rG-CSF and rGM-CSF treatment for neonates with neutropenia.

107.3 Leukocyte Growth Factors in Severe Chronic Neonatal Neutropenia

107.3.1 Kostmann Syndrome

Table 107.1 lists varieties of neutropenia that are generally considered as part of the SCN syndrome. The condition is the result of mutations in the *ELA2* (neutrophil elastase) gene [24–27]. Although rG-CSF treatment is effective in increasing blood neutrophils and reducing febrile illnesses, it does not

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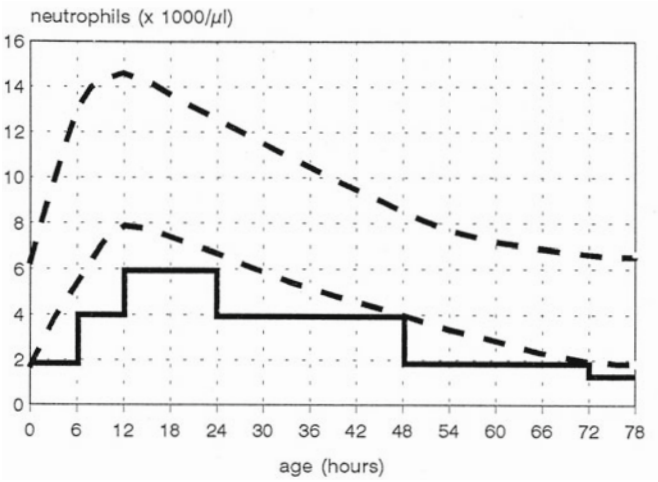
Birthweight > 1500 g

Age (hours)	Neutropenia
0–6	< 2000/μL
>6–12	< 4000/μL
>12–24	< 6000/μL
>24–48	< 4000/μL
>48–72	< 2000/μL
>72	< 1500/μL



Birthweight ≤ 1500 g

Age (hours)	Neutropenia
0–6	< 500/μL
>6–12	< 1500/μL
>12–30	< 1800/μL
>30–48	< 1500/μL
>48	< 1100/μL

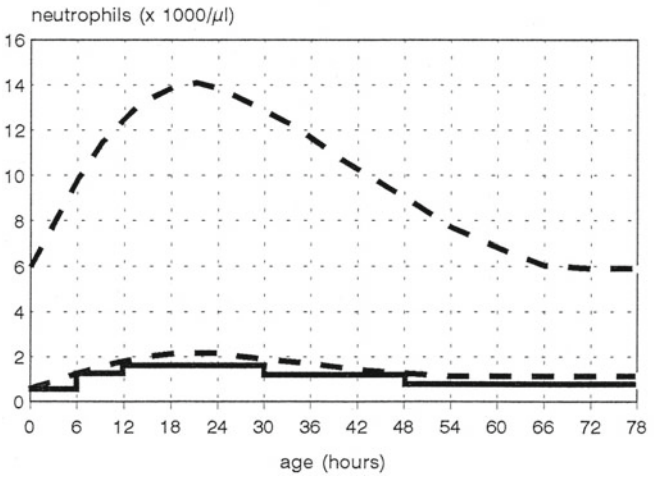


Fig. 107.1 Definitions of neutropenia

Table 107.1 Varieties of neutropenia among neonates that are generally considered SCN

Kostmann syndrome
Shwachman-Diamond syndrome
Barth syndrome
Cartilage-hair hypoplasia
Cyclic neutropenia
Glycogen storage disease type 1b
Severe neonatal immune-mediated neutropenias

generally correct the gingivitis that is a prominent feature of this condition in some families. This is probably because rG-CSF does not increase the natural antimicrobial peptide (LL-37) deficiency in these patients [28, 29].

107.3.2 Shwachman-Diamond Syndrome

This variety of SCN is generally diagnosed after manifestations of exocrine pancreatic insufficiency, with diarrhea and failure to thrive. It is generally an autosomal recessive condition. Some children with this syndrome respond favorably to rG-CSF, yet some progress to bone marrow failure and require marrow transplantation [30, 31].

107.3.3 Barth Syndrome

These patients are generally males (X-linked) with dilated cardiomyopathy, organic aciduria, growth failure, muscle weakness, and neutropenia [32]. rG-CSF can be helpful in these patients as an adjunct to treating infections, or as a preventive measure if their neutropenia is sufficiently severe [33, 34].

107.3.4 Cartilage-Hair Hypoplasia

This is a form of short-limbed dwarfism associated with frequent infections. These patients have short pudgy hands, redundant skin, and hyperextensible joints in the hands and feet but flexor contractions at the elbow. Neutropenia occurs in some patients with cartilage-hair hypoplasia and these can benefit from rG-CSF administration [35].

107.3.5 Cyclic Neutropenia

This condition is caused by mutation in the *ELA2* (neutrophil elastase) gene, and results in periodic drops in blood neutrophil concentration, generally on a three to four-week cycle [36, 37]. Counts can drop to < 500/μL or lower, and infections can be a periodic problem. Because it generally takes several cycles before the diagnosis is considered, most cases are not diagnosed as neonates. rG-CSF administration is useful in preventing the very low nadir counts and thereby preventing infectious complications [36–38].

107.3.6 Glycogen Storage Disease Type 1b

Von Gierke disease is an autosomal recessive disorder caused by a deficiency of the enzyme glucose-6-phosphate translocase,

which transports glucose-6-phosphate into the endoplasmic reticulum for further metabolism. In GSD-1b, glucose-6-phosphate accumulates intracellularly. Affected neonates present with hypoglycemia, hepatomegaly, growth failure and neutropenia. Patients with GSD-1b have recurrent bacterial infections, oral ulcers, and inflammatory bowel disease. The gene causing GSD-1b is located on chromosome 11q23 [39]. rG-CSF can help these patients avoid the recurrent bacterial infections that are otherwise a problematic part of the condition.

107.3.7 Severe Immune-Mediated Neonatal Neutropenia

Most of the severe and prolonged immune mediated neonatal neutropenias are alloimmune [40–43]. However, a few severe and prolonged cases have been found to be autoimmune (maternal autoimmune disease) [43, 44], and a few have been found to be autoimmune neutropenia of infancy (a primary isolated autoimmune phenomenon in neonates) [43, 45].

Alloimmune neonatal neutropenia is a relatively common condition where the mother develops antibodies to antigens present on paternal and fetal neutrophils [40–44]. Antineutrophil antibodies have been found in the serum of as many as 20% of randomly surveyed pregnant and postpartum women [43, 44]. Mostly, such antibodies cause little problem to the fetus and neonate, but up to 2% of consecutively sampled neonates have neutropenia on this basis. This variety of neutropenia can be severe and prolonged, with a median duration of neutropenia of about seven weeks, but a range up to six months. Repeated infections can occur in these patients until their severe neutropenia remits. Delayed separation of the umbilical cord and skin infections are the most common infectious complications, but serious and life-threatening infections can occur. The mortality rate in this condition, due to overwhelming infection, is reported to be 5%. Severe cases have been successfully treated with rG-CSF. Unlike patients with other varieties of SCN, the neutropenia in this condition will remit spontaneously and the rG-CSF treatment can be stopped. Remission occurs when maternal antineutrophil antibody in the neonate has dropped significantly.

Neonatal autoimmune neutropenia occurs when mothers have autoimmune diseases, and their antineutrophil antibodies cross the placenta and bind to fetal neutrophils. Clinical features are generally milder than in alloimmune neonatal neutropenia and it is rare that a neonate with this variety of neutropenia needs rG-CSF treatment.

Autoimmune neutropenia of infancy is an unusual disorder where the fetus, and subsequently the neonate, has a primary isolated autoimmune phenomenon [46–50]. Neutrophil specific antibodies are found in the neonate's serum, reactive against his/her own neutrophils, but no antibodies are found in the mother's serum. Most cases occur in children between three and 30 months of age, with a reported incidence of 1:100,000 children. Affected children most often present with minor infections. Bux reported 240 cases and reported that 12% presented with severe infections, including pneumonia, sepsis, or meningitis [48]. The neutropenia in this condition generally persists much longer than in cases of alloimmune neutropenia, with a median duration of about 30 months and a range from 6–60 months [49, 50]. This variety of neutropenia can be severe, with blood neutrophil concentrations often < 500/μL. rG-CSF administration can increase the neutrophil count and reduce infections complications [48, 50].

107.4 Leukocyte Growth Factors in Neonatal Neutropenia not Categorized as SCN

107.4.1 Pregnancy Induced Hypertension

This is the most common variety of neutropenia seen in the NICU [51–58]. Perhaps 50% of neonates born to mothers with PIH have this variety of neutropenia. The ANC can be very low, frequently < 500/μL, but the count generally rises spontaneously within the first days, and is almost always > 1000/μL by day three. Usually no leukocyte left shift is seen, and no toxic granulation, Dohle bodies, or vacuolization are present in the neutrophils. It is not clear whether this variety of neutropenia predisposes neonates to acquire bacterial infection. Usually the condition is so transient that such a predisposition is unlikely. The condition is probably caused by an inhibitor of neutrophil production of placental origin that might function mechanistically by depressing natural G-CSF production [51–53].

107.4.2 Severe Intrauterine Growth Restriction

This variety of neonatal neutropenia seems to be identical to that associated with PIH. We observed no difference in the onset, duration, or severity of neutropenia in SGA neonates versus neonates born after PIH [59]. Obviously, some neonates born after PIH are also SGA, and it might be that the

Table 107.2 Varieties of neutropenia among neonates that are NOT classified as SCN

Pregnancy induced hypertension
Severe intrauterine growth restriction
The twin-twin transfusion syndrome
Rh hemolytic disease
Bacterial infection
Necrotizing enterocolitis
Chronic idiopathic neutropenia of prematurity

most severe neutropenias in this category are among those with both PIH and SGA. We assume that the neutropenia of PIH and SGA are mechanistically similar and that both are transient with few clinical consequences and no need for rG-CSF administration.

107.4.3 The Twin-Twin Transfusion Syndrome

The donor in a twin-twin transfusion is generally neutropenic, but the recipient can also have neutropenia, although usually not as severe [60]. As with the varieties of neutropenia accompanying PIH and SGA, there is generally no leukocyte left shift nor are there neutrophil morphological abnormalities. This condition is also transient, with the ANC generally spontaneously rising to >1000/μL by 2–3 days, and thus no rG-CSF administration is warranted.

107.4.4 Rh Hemolytic Disease

Neonates with anemia from Rh hemolytic disease are almost always neutropenic on the first day of life [61]. This variety of neutopenia is similar to that of PIH/SGA and donors in a twin-twin transfusion, and is likely due to reduced neutrophil production. The neutropenia is transient, generally resolving in a day or two, and thus no specific treatment is generally required for the neutropenia.

107.4.5 Bacterial Infection

Two strategies have been proposed for rG-CSF usage during neonatal infections.

Since neutropenia commonly accompanies overwhelming septic shock in neonates, perhaps rG-CSF might be a reasonable adjunct to antibiotics and intensive care treatment. Second, since neutrophil function, particularly chemotaxis, is immature among neonates, perhaps rG-CSF administration might be a reasonable way to prevent nosocomial infections among high-risk neonatal patients.

Animal models for both potential uses of rG-CSF were established and supported these hypotheses. In a Cochrane review, Carr et al examined both potential uses [62]. They located seven studies where infected neonates were treated with rG-CSF versus placebo [62–68] and three studies where rG-CSF versus placebo was used as prophylaxis against infections [69–70]. They found no evidence that the addition of rG-CSF or rGM-CSF to antibiotic therapy in preterm infants with suspected systemic infection reduces immediate all-cause mortality. No significant survival advantage was seen at 14 days from the start of therapy [typical RR 0.71 (95% CI 0.38, 1.33)]. They conducted a subgroup analysis of 97 infants from three of the studies who, in addition to systemic infection, had a low neutrophil count (<1700/μL) at trial entry. This subgroup did show a significant reduction in mortality by day 14 [RR 0.34 (95% CI 0.12, 0.92); RD –0.18 (95% CI –0.33, –0.03); NNT 6 (95% CI 3–33)].

The three prophylaxis studies [70–72] did not show a significant reduction in mortality in neonates receiving rGM-CSF [RR 0.59 (95% CI 0.24, 1.44); RD –0.03 (95% CI –0.08, 0.02)]. The identification of sepsis as the primary outcome of prophylaxis studies has been hampered by inadequately stringent definitions of systemic infection. However, data from one study suggest that prophylactic rGM-CSF might provide protection against infection when given to preterm infants who are neutropenic [72].

Carr et al concluded that there is currently insufficient evidence to support the introduction of either rG-CSF or rGM-CSF into neonatal practice, either as treatment of established systemic infection to reduce resulting mortality, or as prophylaxis to prevent systemic infection in high-risk neonates. This conclusion is consistent with other meta-analyses and reviews [71–83].

107.4.6 Necrotizing Enterocolitis

Neutropenia is relatively common among severe cases of NEC. In some cases the neutropenia is transient and resembles the neutropenia following endotoxin [84, 85]. No studies have focused on using rG-CSF among neutropenic neonates with NEC.

107.4.7 Chronic Idiopathic Neutropenia of Prematurity

Certain preterm neonates develop neutropenia when 4–10 weeks old. This variety of netropenia is often associated with a patient's spontaneous recovery from the anemia of prematurity. Neutrophil counts are generally <1000/μL but rarely <500/μL [86–89]. The condition is transient, lasting a few weeks to perhaps a month or more. It appears to be a hyporegenerative neutropenia, because it is not accompanied by a leukocyte left shift nor morphological abnormalities of the neutrophils.

Patients with this condition have an "rG-CSF mobalizable neutrophil reserve", meaning that if rG-CSF is given, their neutrophil count increases within hours. This has been taken as evidence that these patients do not have a significant host-defense deficiency and they can supply neutrophils to tissues when needed [87]. Thus, although these patients can be neutropenic for several weeks, this condition is likely benign and needs no treatment.

107.5 The Use of Leukocyte Growth Factors

G-CSF and GM-CSF are naturally occurring proteins involved in proliferation and differentiation of myeloid precursors into mature neutrophils [90]. The genes for both have been cloned, and the purified recombinant factors are commercially available in pharmacologic quantities [91]. These factors have been widely used in adult and pediatric medicine primarily to treat iatrogenic neutropenia and bone marrow failure syndromes [91, 92]. They can shorten the duration of neutropenia after chemotherapy for leukemia and solid tumors and after bone marrow transplantation. G-CSF is used in patients undergoing peripheral blood hematopoietic stem cell collection. Target cells for these factors differ. G-CSF is lineage specific for the committed progenitors of neutrophils [93, 94]. It stimulates proliferation and differentiation, expanding the available pool of neutrophil precursors and shortening their transit time through the marrow. It has been reported that G-CSF enhances several functions of mature neutrophils, but this has been questioned [95]. GM-CSF acts on multilineage progenitors and on those of monocyte and neutrophil lineage and enhances bactericidal activities of mature phagocytes [90, 91].

Patients with SCN generally derive considerable benefit from rG-CSF administration. Almost all respond to doses of 5–10 micrograms per kg administered at intervals ranging from every day to once per week in order to achieve neutrophil concentrations above 500–1000/μL [90–94].

Explanted blood monocytes and mononuclear cells from human neonates produce G-CSF and GM-CSF poorly, compared with cells from adults. Moreover, cells from preterm infants produce these proteins more poorly than do cells from term infants [95, 96]. Plasma concentrations of G-CSF are relatively low in newborn infants with neutropenia and in infants with presumed sepsis [97, 98]. Neonatal hematopoietic progenitor cells are equally responsive as adult bone marrow progenitors to the actions of G-CSF and GM-CSF in culture. The rapid neutrophil response to infection is impaired in the murine model of G-CSF gene disruption [98]. Animal studies demonstrated diminished production of G-CSF by neonates compared with adults during bacterial sepsis [100, 101]. rG-CSF and rGM-CSF administration in animal models of neonatal sepsis have documented improved survival [100–102].

The decision regarding whether to administer rG-CSF to a neutropenic infant must consider risks and benefits. Animal studies suggesting benefits administered the hematopoietic growth factor concurrently or within hours of inoculation with the bacterial agent. Under the best of circumstances treatment of clinical sepsis with rG-CSF or rGM-CSF within hours of bacterial invasion may not be feasible. In some infants with overwhelming sepsis bone marrow neutrophil storage pools may already be depleted, thus limiting the capability for response to rG-CSF or rGM-CSF treatment. Additionally, septic and neutropenic infants exhibit elevated circulating and urinary G-CSF concentrations suggesting that their G-CSF receptors are saturated with endogenous G-CSF [103].

The risk of developing nosocomial infection in preterm neonates with neutropenia due to maternal hypertension is controversial [104]. Some studies have demonstrated an increased incidence of nosocomial infections in these neonates, but others have not. Studies evaluating rG-CSF administration to correct the neutropenia caused by maternal hypertension have demonstrated a significant increase in circulating neutrophils compared with study entry, but have not demonstrated lower rates of infection [105, 106].

rGM-CSF has been studied in neonates using slightly different strategies than used in trials of rG-CSF administration. Three prophylaxis studies using rGM-CSF have been published. These studies adopted different treatment regimens and did not use comparable criteria for identifying episodes of systemic infection. rGM-CSF increased the circulating absolute neutrophil count and bone marrow neutrophil storage pool. Neutrophil function was enhanced as defined by up-regulation of surface C3bi expression [70, 71]. Circulating monocytes and platelet counts were increased among rGM-CSF recipients. A systematic review of these studies concluded that rGM-CSF prophylaxis did not lead to a significant reduction in mortality [62].

Similarly, a recent multicenter randomized controlled trial of prophylactic rGM-CSF for extremely preterm infants at high risk of sepsis did not demonstrate reductions in sepsis or improved survival [107].

107.6 A Consistent Approach to the Use of rG-CSF and rGM-CSF in the NICU

Fig. 107.2 is a simple algorithm for making the decision regarding rG-CSF administration in the NICU. It is intended as a guideline to serve until sufficient data accumulate for an evidence-based change in this approach. The algorithm is in

Table 107.3 Screening for severe chronic neutropenia

Inclusion questions:

1. Has a blood neutrophil count of <500/μL been documented on at least three occasions in the past three months?
2. Is there a history of recurrent infections? (specify)
3. Is the bone marrow evaluation consistent with severe chronic neutropenia? (date performed)
4. Has a cytogenetic evaluation been completed?
5. Is the patient now receiving Neupogen (rG-CSF)?

Exclusion criteria

1. The neutropenia is known to be drug-induced
2. Thrombocytopenia is present (<50,000/μL) except in the case of Shwachman-Diamond syndrome or glycogen storage disease type 1b
3. Anemia is present (hgb <8g/dL) except in the case of Shwachman-Diamond syndrome or glycogen storage disease type 1b
4. The patient has a myelodysplastic syndrome, aplastic anemia, is HIV positive, has some other hematological disease, has rheumatoid arthritis, or has had previous chemotherapy for cancer

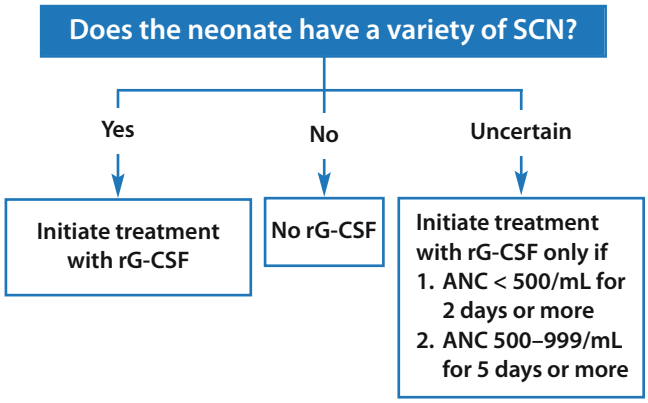


Fig. 107.2 The US FDA approved rG-CSF for use in children with severe chronic neutropenia, and for children receiving myelosuppressive chemotherapy of marrow transplantation. The rG-CSF used in the USA is produced by Amgen (Thousand Oaks, CA) and has a molecular weight slightly lower than natural G-CSF, because it is produced in *Eschericha coli* and is not glycosylated. It has an amino acid sequence identical to that of natural G-CSF except for the addition of an N-terminal methionine, which is necessary for its expression in *E. coli*. SCN includes Kostmann syndrome, Shwachman-Diamond syndrome, cyclic neutropenia, Bart syndrome, autoimmune or alloimmune neonatal neutropenia, or any syndrome or condition where severe and chronic neutropenia are a consistent finding. G-CSF administration is not recommended for neonates with neutropenia of a non-severe, or a non-chronic nature, such as the neutropenia associated with PIH, sepsis, or chronic idiopathic (mild) neutropenia

keeping with FDA approved approaches and avoids off-label uses. The principal question is whether the neutropenic neonate has a variety of SCN; a question to which there are three possible answers; 1) Yes, 2) No and 3) Uncertain. We propose that if the answer is yes, the patient should be afforded the benefit of rG-CSF treatment, and if the answer is no, rG-CSF should not be given. Cases where the answer is uncertain, might receive rG-CSF if the neutropenia is severe (ANC <500/μL for 2 days of more) or prolonged (<1000/μL for 5 days or more) while the question of SCN is being actively investigated.

An international registry for SCN cases can be accessed at the website http://depts.washington.edu/registry/, using the entry criteria and exclusion criteria given in Table 107.3. We propose beginning treatment with a dose of 10 μg/kg subcutaneously, once per day for three consecutive days. Thereafter doses are given as needed to titrate the ANC to around 1000/μL. We did not include criteria for administering rGM-CSF, as we found insufficient evidence for its use in the NICU. If one follows this schema (Fig. 107.2) it will result in little use of rG-CSF in the NICU. Any rG-CSF usage in Neonatology should be focused on patients with the most to gain and least to lose by its application. As additional pertinent investigative work is published, these guidelines shall be modified accordingly.

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Fundamentals of Feto-Neonatal Immunology and Its Clinical Relevance

Akhil Maheshwari and Edmund F. La Gamma

108.1 Introduction

The transition from fetal to neonatal life at birth forms an important functional watershed in the developing immune system. In utero, the fetus is exposed to a steady stream of foreign antigens that are derived mainly from the mother, and must down-regulate its immune response to survive. After birth, however, the neonatal immune system is exposed to a new, more diverse set of antigens and must evolve dichotomous responses to contain the micro-organisms on various cutaneous and mucosal surfaces, and at the same time, develop tolerance to other commensal microbes and dietary macromolecules. During this remarkable transition, while some components of the immune system perform at par with adults, immaturity of the other arms results in a developmentally-regulated state of immunodeficiency. This chapter highlights major quantitative and qualitative differences in the innate and adaptive arms of the neonatal and adult immune systems and provides a brief review of the developing mucosal immune system.

108.2 Clinical Relevance

Preterm neonates have a high susceptibility to bacterial infections, especially of the Gram positive and Staphylococcal variety suggesting a preponderance of vulnerability due to neutrophil function. They are also remarkably deficient in circulating antibody after their trans-placental passage, yet preterm neonates can generate an amnesic response to produce antibodies. Nevertheless, this makes them vulnerable to impaired opsonization (a bacterial vulnerability) and viral dissemination (an uncommon but real biological threat). How these vulnerabilities arise and what opportunities remain to augment defenses can be understood best by an in depth grasp of how the mature capacities of function change over time. Our goal is to highlight those features.

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108.3 Innate Immune System

This section will review the developmental issues relevant to the collection of defense mechanisms the human neonate is born with prior to exposure to environmental antigens and acquisition of adaptive immunity. Cell lines, their maturation and functional capacities or limitations will be defined.

108.3.1 Neutrophils

108.3.1.1 Development

The two major hematopoietic progenitors committed to the neutrophil lineage are the colony-forming units (CFU)-mix, which give rise to a mixture of various leukocyte populations, and the CFU-GEMM, which produce granulocytes, erythrocytes, megakaryocytes, and macrophages. The mechanisms underlying this process of lineage commitment are now beginning to be understood [1]. The effect of biomechanical forces as critical regulators of hematopoiesis is a new field of investigation [2], as is the prevention of apoptosis as a mechanism for hematopoietic progenitor cell growth and development [3]. In the bone marrow, the neutrophil cell lineage includes the early precursors with a capacity for 4–5 five cell divisions, and the later, post-mitotic stages that are in the process of differentiation (Fig. 108.1). In adults, the neutrophil proliferating pool (NPP) contains about 2×10^9 cells/kg body weight and the neutrophil storage pool (NSP) contains about 6×10^9 cells/kg body weight [4]. The NPP and NSP together contain nearly 90% of all neutrophils in the

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

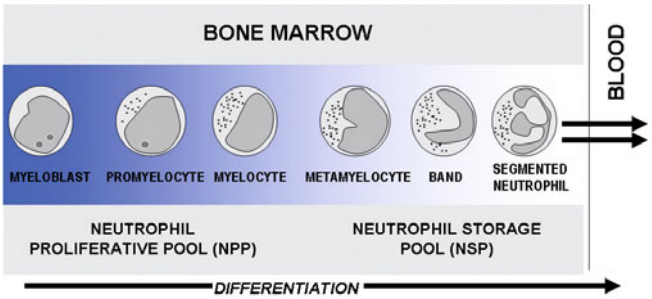

Fig. 108.1 Neutrophil differentiation process in bone marrow

body. The remaining 10% (0.6×10^9/kg) are equally distributed free in the circulation or attached to the microvascular endothelium.

Although umbilical cord blood and fetal blood have a 10–50 fold higher concentration of CFU-GM than does the blood of adults [5], the fetus has an overall much smaller pool of neutrophil progenitors [6]. In the mid-gestation fetus and preterm infant, the NSP is very small in size and can be readily exhausted during sepsis [4]. Studies in experimental animals show that the NSP is considerably smaller in animals delivered prematurely ($1.0–1.3\times10^9$ cells/kg) than at full-term ($1.3–2.5\times10^9$ cells/kg) and in adulthood ($4.5–7.5\times10^9$/kg). The NPP is also smaller, about one-tenth the size (per kg body weight) of adults. Indirect evidence for a small granulocytopoietic pool in the human fetus come from studies of soluble CD16, which is derived from apoptotic neutrophils, and its low concentrations in plasma of preterm neonates may reflect a low total body neutrophil mass. The low sCD16 levels of premature infants reach adult values only by the fourth postnatal week [7].

During acute inflammation, neutrophils are released from the NSP first, and once these stores are exhausted, progressively immature cells are mobilized (known as the left shift of sepsis). Circulating neutrophils persist in the bloodstream for 6–8 hrs and then for a few hrs to several days in tissues. In the fetus, as in the adult, mature neutrophils are stored within the bone marrow and also in the liver and spleen. These maturational relationships and short-half life frequently result in the presenting clinical sign of neutropenia rather than neutrophilia in newborns with bacterial sepsis.

108.3.1.2 Function

Transendothelial Migration

Circulating neutrophils leave the intravascular compartment to enter the tissues in three major steps: margination and rolling on vascular endothelium, attachment to the endothelial cells, and transendothelial migration [8]. Leukocyte traffic is preferentially directed to inflamed areas through regional changes in vascular flow and along concentration gradients of humoral chemoattractants such as chemokines, bacterial products (such as formyl-met-leu-phe or f-MLP), complement fragments (C5a), and leukotrienes (LTB_4). Among chemokines, members of the CXC subfamily with a glutamate-leucine-arginine (ELR) tripeptide sequence (such as interleukin-8 (IL-8/CXCL8)) have a neutrophil-specific chemotactic activity [9, 10].

In inflamed tissues, transient interruptions in the laminar flow of marginating neutrophils cause these cells to tumble and roll on the vascular endothelial surface. This process is mediated through a process of repetitive binding and release of selectins (L-selectin on neutrophils, E- and P-selectin on endothelium) from their sialomucin receptors called addressins. The term selectin is derived from a key lectin domain that interacts selectively with oligosaccharide receptors bearing sialylated carbohydrate moieties. L-selectin is constitutively expressed on neutrophils and is shed after cellular activation [11]. Rolling neutrophils slow down and attach to endothelium through the binding of β_2-integrins to cognate receptors on endothelial cells [12]. The β_2-integrins expressed on neutrophils include the leukocyte function-associated antigen-1 (LFA-1, $\alpha_L\beta_2$, CD11a/CD18), Mac-1 ($\alpha_M\beta_2$, CD11b/CD18), and the p150, 95 ($\alpha_X\beta_2$, CD11c/CD18), which bind endothelial receptors such as the intercellular adhesion molecule-1 (ICAM-1) and ICAM-2. LFA-1 binds to both ICAM-1 and ICAM-2, whereas Mac-1 and p150, 95 bind exclusively to ICAM-1. These neutrophils undergo activation on the endothelial surface and migrate through the capillary/venular wall, a process that involves the platelet-endothelial cell adhesion molecule (PECAM1, CD31), the integrin-associated protein (CD47), and a series of other junctional molecules [13].

Compared to neutrophils from adults, neutrophils from both term and preterm infants adhere poorly to the endothelium. Neonatal neutrophils have lower selectin expression than adults at birth [14], which might be further reduced by perinatal stress such as in birth asphyxia [15]. In addition, neonatal neutrophils display defective shedding of L-selectin [14]. This combination of decreased expression and impaired shedding of L-selectin impairs the ability of neutrophils to roll on the endothelial surface, thereby limiting the recruitment of circulating neutrophils into the tissues. Neutrophil-endothelial adherence and neutrophil transmigration is further limited in neonates due to a developmental deficiency of Mac-1 (CD18/CD11b), one of the β_2 integrins [16]. The transendothelial migration of neutrophils is also limited, at least in immature cells released from the NSP during sepsis, by reduced deformability of the neutrophil membrane and cytoplasm [17]. For these reasons, it is generally believed that the propensity of neonates to bacteremia is more typical than a proper localization and abscess formation.

Chemotaxis

Once outside the blood vessel, neutrophils migrate along concentration gradients of various chemoattractants such as IL-8 (and other ELR^+ CXC chemokines), f-MLP, and C5a [9].

These chemotactic stimuli bind to high-affinity G-protein-coupled receptors on the leukocyte surface, and minute spatial gradients in chemoattractant concentrations can cause the receptors to be distributed asymmetrically towards the migrating neutrophil pseudopodium. Cellular movement involves a number of intracellular signaling pathways and cytoskeletal proteins. A chemoattractant hierarchy has been reported wherein bacterial products are preferred over host chemokines [18].

Neutrophils from both term and preterm neonates have an impaired chemotactic response, migrating at only about half the speed traveled by adult cells [19, 20]. While neutrophils from term infants achieve normal chemotactic function by 2 wks after birth, such postnatal neutrophil maturation begins 2–3 weeks after birth in immature preterm infants and proceeds very slowly [21]. Neutrophils from preterm infants born at 34–36 wks gestation achieve normal chemotaxis by 40–42 weeks of post-conceptional age. In more immature preterm infants (<34 wks), neutrophil chemotaxis improves with time, but remains impaired in comparison to adults even at 42 wks PCA [22]. Although minor infections may enhance chemotaxis in neonates, the migratory responses of neonatal neutrophils may become further depressed during systemic Gram-negative sepsis [23].

Neonatal neutrophils bind various chemoattractants normally. However, chemoattractant-induced membrane depolarization, calcium transport, and sugar uptake are relatively less efficient. The chemotactic defect in neonatal neutrophils may be multi-factorial, affected by factors such as a larger, poorly motile neutrophil subpopulation, impaired calcium mobilization, and aberrations in intracellular signaling pathways such as NF-κB activation [24, 25]. Lower Mac-1 expression can also impede chemotaxis due to impaired neutrophil interaction with the extracellular matrix [24]. Inability to effectively direct neutrophils to the bacterial source contributes to the neonatal vulnerability to septicemia.

Phagocytosis

Phagocytosis is a specialized form of endocytosis directed at engulfing solid particles into an internal phagosome. This internalized phagosome "matures" through interactions with the endosomal compartment and eventually fuses with a lysosome for killing of internalized microorganisms and terminal degradation of the cargo [26]. Phagocytosis is more efficient when the target is opsonized by specific immunoglobulin G (IgG) or complement factors, which may act by neutralizing inhibitors of phagocytosis such as the capsular polysaccharide or by rendering the microbial surface more hydrophobic. Neutrophils express receptors for IgG ($F_c\gamma$ receptors I-III, or CD16, CD32, CD64), C3b (CR1), and iC3b (CR3). In some instances, microorganisms may be ingested without opsonization through lectin-carbohydrate (lectins on bacterial fimbriae interact with neutrophil glycoproteins), protein-protein (proteins such as filamentous hemagglutinin that express the arg-gly-asp or the RGD amino acid sequence bind to integrins), and hydrophobic-protein (bacterial glycolipids and neutrophil integrins) interactions [26].

The interaction of IgG or complement receptors on the neutrophil surface with the opsonized particle trigger cytoskeletal rearrangements to enclose the opsonized particle within a phagosome. Phagocytosis is most efficient when organisms are coated with both IgG and C_3, which allows cooperative interaction of cognate receptors for both the opsonins. As mentioned above, neutrophils express integrin receptors for matrix proteins with the RGD tripeptide motif (such as fibronectin, laminin, and collagen), and ingest C_3-coated particles more efficiently when adherent to surfaces coated with these RGD-bearing proteins [26, 27].

Preterm neutrophils have impaired phagocytosis, which corrects only in the late third trimester to become comparable to adults [19]. Preterm neutrophils ingest particles more slowly and ingest fewer bacteria (such as *E. coli*). The lack of opsonic activity is an important consideration, as preterm infants often have lower concentration of specific antibodies [28]. Compared to term neonates and adults, preterm neutrophils also have decreased expression of CD16 ($F_c\gamma$RIII) and CD32 ($F_c\gamma$RII), the two most abundant neutrophil IgG receptors [29]. Whereas CD16 expression normally increases to adult levels over the first three weeks of life, CD32 deficiency may or may not correct with time [30].

Intracellular Killing

The phagolysosome provides an enclosed space in which an ingested microbe is exposed to high concentrations of toxic substances, while limiting the exposure of the phagocyte and other cells to these potentially injurious agents [26]. The major killing mechanism in neutrophils involves the generation of reactive oxygen species (ROS) in a respiratory burst. An NADPH-dependent oxidase localized on the cell membrane (and therefore, the phagosome membrane) reduces molecular oxygen (O_2) to a superoxide anion ($O^{\bullet}_2^-$) [31]; subsequent generation of peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$, formed in the presence of iron) also contributes to the microbicidal capacity of neutrophils [32]. These oxygen-dependent bactericidal mechanisms can be broadly divided into myeloperoxidase (MPO)-independent (such as hydrogen peroxide) and MPO-dependent (MPO catalyzes reactions between H_2O_2 and halides to form highly reactive products) [33]. H_2O_2 is a weak bactericidal agent per se, but the MPO-H_2O_2-halide system increases its efficacy by nearly 50-fold. The bactericidal effects of free oxygen radicals are due to oxidizing effects on various components of the bacterial cell wall [32].

Neutrophils also have elaborate non-oxidative killing mechanisms such as low pH (as low as 6.0), defensins, bactericidal/permeability-increasing protein (BPI), lactoferrin, lysozyme, and a variety of cationic proteins. Defensins are

broad-spectrum antimicrobial peptides with activity against gram-positive and gram-negative bacteria, fungi, and enveloped viruses and are also released in the gut by Paneth cells [34]. BPI binds lipopolysaccharide (LPS) and blocks its effects, can damage the outer membrane of gram-negative bacteria, and has some opsonic activity. Lactoferrin, an iron chelator, is bacteriostatic as it deprives bacteria of the iron required for growth. Lactoferrin is also involved in neutrophil degranulation, in oxygen radical production, and in granulocytopoiesis. Lysozyme hydrolyzes a glycoside bond in the bacterial cell wall peptidoglycan. Primary granules also contain other cationic antibacterial proteins such as azuricidin, indolicin and cathelicidins [26, 35].

The respiratory burst is depressed in preterm neutrophils, which explains the observed developmental defects in intracellular killing of bacterial pathogens such as *Staphylococcus aureus* or *E. coli* [36]. The killing capacity cannot be fully explained on the basis of low opsonic activity in preterm plasma and improves only as a function of gestational age [19, 37, 38]. The neutrophil respiratory burst in infants born at 24–28 wks is clearly less robust than in those born at 29–35 wks and takes about 2 months to correct. However, neutrophils from preterm infants continue to have an overall weaker oxidative burst than adults and may not show any improvement in critically-ill preterm infants [39]. Neonatal neutrophils are also less effective in killing group B streptococci, although the data on candidacidal activity are conflicting. The antiviral activity of neonatal neutrophils is also diminished compared with that of adults. Indeed, the most commonly recovered bacteria in extremely low birth weight/extremely low gestational age neonates (ELBW/ELGAN) is a commensal organism *Staphylococcus epidermidis*.

Degranulation

Neutrophils contain two major types of granules (Fig. 108.2): (1) the azurophilic granules (stain positive with the azure A dye) and (2) specific granules (do not stain with azure A). Azurophilic granules contain myeloperoxidase (MPO), proteolytic enzymes such as cathepsins, proteinase-3, and elastase, and antimicrobial proteins such as defensins and the bactericidal permeability-increasing protein (BPI). These granules release their contents into the phagolysosomes and are involved in intracellular killing. The specific granules contain antibacterial agents such as lactoferrin and lysozyme, receptors for complement components, and bacterial products such as f-MLP. Specific granules fuse with the cell membrane to release their contents by exocytosis, and also bring functionally important membrane proteins such as integrins, cytochrome-b_{558}, and receptors for chemotactic agents and opsonins to the cell surface. Specific granules play an important role in extracellular killing [40].

Neutrophils from term neonates have granule contents and degranulation responses similar to adults [41]. However, neutrophils from preterm infants have a lower capacity to release BPI, elastase, and lactoferrin than in adults and term infants [19, 42]. Collectively, neutrophil line immaturities and limited function accounts for a substantial component of neonatal susceptibility to invasive bacterial infections.

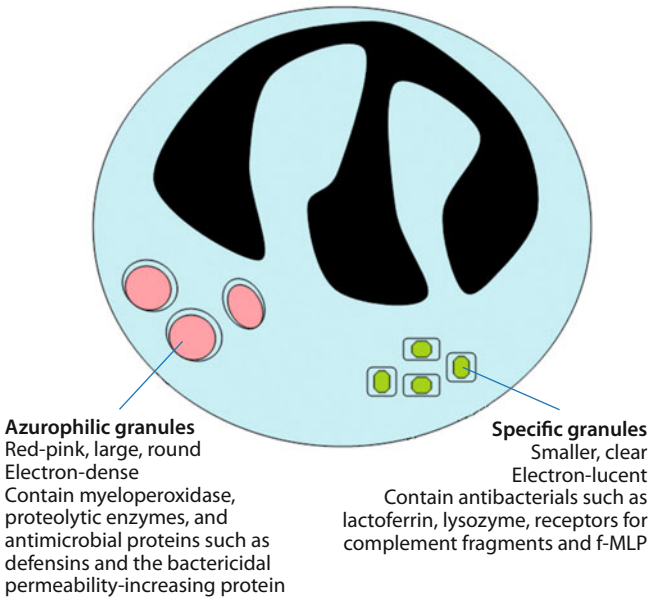

Fig. 108.2 Types of granules in neutrophils

108.3.2 Monocytes and Macrophages

108.3.2.1 Development

Embryonic macrophages are first seen in the yolk sac at 3–4 weeks of gestation. Unlike macrophages in the fetus and the adult, which are derived from circulating monocytes, these large-sized histiocytic cells develop in the early embryonic period from yolk sac progenitors prior to the first appearance of monocytes [43, 44]. At 5 weeks of gestation, two distinct cell lineages with a dendritic/macrophage structure can be identified in the yolk sac, mesenchyme and the fetal liver. The larger subgroup is MHC II-negative, and there is only a minor population that expresses these antigens. MHC II-negative cells also appear in the thymic cortex, in the marginal zones of lymph nodes, in the splenic red pulp, and in the bone marrow [45]. A few MHC II-positive cells are seen in the liver at 7–8 weeks of gestation, the lymph nodes at 11–13 weeks of gestation, and the T-cell areas of the developing thymic medulla by 16 weeks of gestation. Subsequently, the numbers increase gradually, and MHC class II-positive cells are also seen in the skin and gastrointestinal tract [45].

During the second month of gestation, as hematopoiesis becomes established in the fetal liver, monocytes are seen in

high proportions and constitute nearly 70 percent of all hematopoietic cells [46]. Over the next 6 weeks, as the erythroid cells predominate, this proportion falls to 1–2 percent [46]. The first monocytes in circulation are not seen until about the fifth month of gestation [46], and remain uncommon until the bone marrow becomes the predominant site of hematopoiesis [47]. At 30 weeks, monocytes comprise 3–7 percent of hematopoietic cells [48]. Term cord blood studies show a relative monocytosis, which persists during the neonatal period. The absolute monocyte counts tend to decline gradually from 1340–2200/μL in the first week to about 700 in the third week [49]. We are currently analyzing data from a large cohort of neonates with more than 100,000 blood counts to define the normal ranges of absolute monocyte counts.

Information on tissue macrophage kinetics in the neonatal period is mainly from autopsy studies. The size of the macrophage pool varies in different organ systems. In the gastrointestinal tract, macrophages appear in the lamina propria as early as 10 weeks of gestation and a sizable macrophage population can be seen during midgestation [44]. In contrast, the alveolar macrophage population remains small in the fetus and expands rapidly during the early neonatal period [50]. This increase may result both from an influx of monocytes from the circulation as well as from clonal expansion in situ.

Monocytes and macrophages can be identified using immunocytochemistry for the macrophage marker HAM56, cellular constituents such as nonspecific esterase and peroxidase, innate immune receptors such as CD11b, and the MHC complex [51–54]. A lower proportion of cord blood macrophages stain for esterase, although there are no discernible differences in peroxidase activity [52]. Neonatal monocytes have lower expression levels of the aforementioned surface markers, except for CD14 [53]. In certain tissues, such as in the gastrointestinal tract, resident macrophages are characteristically downregulated for the expression of innate immune receptors such as CD14 [55].

Due to the extraordinary importance in this particular lineage in epithelial surface barrier protection, the propensity of ELBW/ELGAN patients to pneumonias, necrotizing enterocolitis and invasive skin infections merits consideration.

108.3.2.2 Function

Transendothelial Migration, Chemotaxis, Phagocytosis, and Respiratory Burst

Unlike neutrophils, the major host defense functions of monocytes in cord blood of term infants are largely intact. Cord blood monocytes show adherence, random migration, chemotaxis, bactericidal activity, phagocytosis-associated chemiluminescence, production of superoxide anion (O-2) and generation of hydrogen peroxide at levels comparable to those of cells from healthy adult volunteers [56, 57]. The ability of fetal and neonatal monocytes to kill a variety of pathogens including *S. aureus*, *S. epidermidis*, *E. coli*, and *C. albicans* appears to be equivalent to that of adults [56, 58].

Resident macrophages are often the first phagocytic cells of the innate immune system to encounter invading pathogens breaching the various epithelial surfaces of the skin, gut and lung. These cells serve important host defense functions through phagocytosis, and also as sentinel cells that regulate local inflammatory responses by producing various cytokines and chemokines [59, 60]. Most studies on monocyte/macrophage cell function have been done on cord blood, and fetal cells have not been studied to the same extent so far. Term cord-blood monocytes produce IL-1, IFN-α and TNF-α in concentrations that are comparable to adults, but the levels of IFN-γ, IL-8, IL-10, and GM-CSF are lower. These cells also produce lower concentrations of extracellular proteins like fibronectin, and bioreactive lipids like leukotriene B_4 [61, 62]. Impaired monocyte secretory functions in neonates may be partially responsible for poorer cytokine responses of neonatal T-cells.

Emerging evidence indicates that macrophages are dynamic and heterogeneous cells, which are polarized into the classically-activated M1 macrophages that express various inflammatory signals, and the more-recently described M2 macrophages that function with an anti-inflammatory profile [63]. Although the effect of development on macrophage polarization is not known, the relative inability of cord blood monocytes (versus monocytes from adults) to mount a robust inflammatory cytokine response is intriguing [64].

108.4 Adaptive Immune System

108.4.1 Dendritic Cells

108.4.1.1 Development

Dendritic cells (DCs) are a discrete leukocyte population with a highly developed antigen-presenting function. DC populations have been grown from separated hematopoietic precursors, suggesting that there is a common granulocyte-monocyte-dendritic cell progenitor [65]. Cells with a dendritic/macrophage structure are present in the yolk sac, mesenchyme and the liver at 4–6 weeks of age. DCs are detectable in skin by 6–7 weeks of gestation.

DCs initially derived their name from their distinctive morphology, with numerous fine dendritic cytoplasmic processes commonly found penetrating epithelial bound organ surfaces. However, phenotype alone is not sufficient to define these cells in view of functional differences between the subpopulations. A working definition requires DCs to be able to stimulate T-cells, home to T-cell dependent lymph node areas, be able to pinocytose and have characteristic cell surface antigens.

Human peripheral blood DCs mainly include two subgroups [66]: (1) myeloid DCs (or mDCs) are $CD11c^+$ cells that express myeloid markers such as CD13, CD33, and CD11b; and (2) plasmacytoid DCs (or pDCs) are $CD11c^-$ and have a plasmacytoid morphology with well-developed rough endoplasmic reticulum and Golgi apparatus.

108.4.1.2 Function

Cord blood-DCs represent about 0.3% of all mononuclear cells. Most studies show an increased number and proportion of pDCs in cord blood compared to adult peripheral blood, with pDC:mDC ratios of 1–3:1 that contrast with the usual 1:2 ratio in adults [67].

Due to the low frequency of DCs in peripheral blood, most studies of neonatal DCs have been carried out using *in vitro* monocyte-derived dendritic cells (MDDCs). Compared to pDCs from adults, cord blood DCs exhibit low or no basal expression of co-stimulatory molecules CD40, CD80 or CD86, show an impaired maturational response following stimulation with agonists for various toll-like receptors (measured as an increase in the expression of co-stimulatory molecules and production of IFN-alpha, TNF-alpha, IL-1, IL-6 and IL-12), and perform poorly at accessory function [68]. Cord-blood dendritic cells have lower expression levels of ICAM-1 and MHC antigens than in adults. These cells are also poor stimulators of mixed lymphocyte reactions, regardless of whether cord or adult mononuclear or T-cells were used as the responders. How precisely these cells contribute to the susceptibility of neonates to maturation of adaptive immunity, prevention of invasive illness and barrier dysfunction is an area in need of further exploration.

108.4.2 T-Lymphocytes

108.4.2.1 Development

The thymus arises at about six weeks of gestation from the third branchial arch, with the cortex arising from its ectodermal layer and the medulla from the endoderm. Lymphoid cells migrate over the next 2–3 weeks, initially from the yolk sac and fetal liver, and then from the bone marrow to colonize the fetal thymus [69]. These prothymocytes interact with the stroma, proliferate actively, and are triggered to differentiate with expression of the first T-cell-specific surface molecules (e.g., CD2, and later CD4 and CD8) [70, 71]. A clear delineation of the thymic cortical and medullary regions occurs at 12 weeks of gestation; Hassall's corpuscles appear shortly thereafter [72, 73]. The most immature thymocytes are found in the subcapsular cortical region, and cells move into the deeper layers as they mature [72].

The early prothymocytes do not express CD3, the T-cell receptor (TCR), CD4, or CD8 and are often referred to as triple-negative thymocytes [74]. The progeny continue to divide and rearrange their TCR genes, and since these cells express both CD4 and CD8, they are now called double-positive [72, 74]. They undergo positive selection by self-major histocompatability complex (MHC) restriction, and more than 95 percent (about 50 million) cells die each day during this stage [74]. Negative selection occurs next, and is mediated by the bone marrow-derived antigen-presenting cells (APC) (e.g., dendritic cells and macrophages), which eliminate autoreactive cells either by clonal deletion or clonal anergy [75]. As these thymocytes mature and reach the medulla, they express only one of the CD4 or CD8 antigens. These single-positive T-cells migrate from the thymus to the peripheral lymphoid organs at about 14 weeks of gestation [72]. By 15 weeks, human thymocytes express a complete set of TCRs [72, 76].

During fetal life, thymus is the largest lymphoid tissue in terms of body proportions. It is about two thirds its mature weight at birth, and reaches its peak mass at around 10 years of age. Subsequently, it continues to involute and is replaced by adipose tissue [77].

108.4.2.2 T-Cell Receptor (TCR) Repertoire

The TCR is composed of two distinct functional subunits, each specialized for a different function [78]. The first, highly polymorphic, is uniquely structured for each T-cell for antigen recognition; it is composed of two polypeptide chains, α and β (except in a specific T-cell subset where it consists of γ and δ chains) [78]. The second, also known as CD3, is a trimolecular complex, involved in signal transduction and cellular activation. The extracellular region of the TCR resembles an immunoglobulin (Ig) Fab fragment, and derives its structural diversity from recombinatorial permutations involving a set each of Variable (V), Diversity (D), and Joining (J) gene segments [79, 80]. The variable domain is situated in the N-terminal end of the α/β (or γ/δ) chains, whereas the C terminal is the constant region [78]. The variable domains consist of V, D, and J elements in the β chain, and V and J in the α chain [81]. The antigen-binding sites are formed by three complementarity-determining regions (CDRs). CDR3, the most extensive of these segments, serves as a key site for antigen recognition [82].

By midgestation, all the TCR Vβ families (V here refers to the variable domain of the β chain, and should be recognized as different from the V gene segments mentioned above) are expressed, but they have shorter CDR3 regions. This is primarily due to limited expression of the enzyme terminal deoxynucleotidyl transferase (Tdt), which induces N-terminal diversity and, consequently, CDR3 heterogeneity. In term infants, the T-cell Vβ repertoire is similar to that seen in adults [82, 83]. Cord blood T-cells from term infants are also able to expand the TCR β repertoire on stimulation with bacterial toxins [83]. It is the central position of T-cell function in the maturing immune system that places the capacity to

adapt to a changing environment central to successful maturation of immune defenses. This is primarily a time and antigen experience-dependent process influenced by the maternal inoculation with flora during a normal birth as well as the subsequent clinical and home environments as influenced by clinicians and their use of antibiotics or other teleologically relevant experiences.

108.4.2.3 Circulating T-Cells

T-cell subpopulations gradually increase in number beyond 19 weeks' gestation, and continue to rise after birth to peak at about 6–9 months. The numbers subsequently decline, and adult levels are finally reached at 6–7 years of age [84]. In term neonates, CD4+ cells constitute a higher proportion of T-cells than adults. CD8+ cells, on the other hand, are fewer both in terms of their absolute number and as a percentage of total T-cells. The CD4/CD8 ratio, consequently, is as high as 4.9:1 during the perinatal period, and declines to adult values of approximately 2:1 only by 4 years of age [84]. Preterms have significantly higher numbers of CD4+ T-cells, but the number of CD8+ T-cells does not seem to change with gestational age [85]. These numbers decrease with perinatal distress, but normalize by 3 weeks of age except in some very-low-birth-weight infants [85].

Peripheral T-cells in the fetus and neonate may be in a relatively immature transitional state; in cord blood, nearly 85% of T-cells express CD38 (compared with less than 5% of adult cells), but lack other markers of activation. Cord blood T-cells also differ in being predominantly naïve (80–90% express the CD45RA phenotype, compared with only 40–60% of the adult cells) [86]. The percentage of memory T-cells (CD45RO) increases in healthy infants during the first few years of life, but reaches adult levels only by the second decade [86]. Neonatal T-cells also have lower expression of CDw29 and CD11b, providing further evidence to a lack of previous stimulation [86]. The ratios and relationships of T-cell subtypes and the clinical relevance to invasive disease is an area of perinatal immunology that is in need of further clarification.

108.4.2.4 Function

Proliferation

Cord blood T-cells from premature infants have a limited capacity for mitogen-induced proliferation but these defects are corrected by full-term [86, 87]. Similarly, proliferative responses to monoclonal antibodies against T-cell markers (e.g., CD3, CD2) are dramatically less than adult lymphocytes, and resemble adult naïve T-cells [88]. These responses improve as the numbers of peripheral blood memory T-cells increase. When tested with allogeneic cells (mixed lymphocyte reaction), however, cord blood lymphocytes respond better, though still somewhat less than cells from adult subjects [86, 87, 89, 90].

Cytokine Production

Neonatal concentrations of pro-inflammatory cytokines like IL-1, IL-6, TNF-α, IFN-α, and IFN-β are comparable to adults, and also increase similarly during sepsis [91, 92]. Premature infants, however, are known to produce lower amounts of TNF-α and IFN-α compared to those born at term [93, 94]. Among the cytokines involved in adaptive immunity, only IL-2 concentrations are comparable; others like IL-4, IL-5, IL-10, IL-15 and IFN-γ are known to be significantly lower than adults [93–96]. Transforming growth factor (TGF)-β_1 and macrophage inflammatory protein-1alpha (MIP)-1α, both of which play a negative role in hematopoiesis, are also present in much lower concentrations [97].

The concentrations of hematopoietic colony stimulating factors including IL-3, Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF), Granulocyte-CSF and Monocyte-CSF are slightly lower to comparable to adults, but even full-term infants are known to be unable to mount adequate responses during stress [98]. Chemokines, which have emerged as important regulators of leukocyte trafficking, are currently an important area of study. The concentrations of some of these are known to be comparable to adults, including IL-8/CXCL8, epithelial neutrophil attractant (ENA)-78/CXCL5, growth-related oncoprotein (GRO)-α/CXCL1, eotaxin/CCL11, and RANTES (regulated upon activation, normal T-cell expressed and secreted)/CCL5 [99].

Most of these deficiencies are caused by altered regulation of post-transcriptional mRNA processing [97]. It is believed that lower concentrations of some of these cytokines are caused by a relative paucity of memory or primed T-cells. However, genetic and other individual traits such as atopy are also likely to be important determinants of the cytokine response [100].

During development, naïve T-cells differentiate into distinct effector T-helper (Th) subsets, a process in which cytokines play a critical role. These differentiated T-cells were originally categorically designated Th1 and Th2 cells based on distinct functional properties and the cytokines that drive their development [101]. Th1 type cytokines, such as IFN-γ and IL-2 play a key role in initiating early resistance to pathogens, and induction of cell-mediated immunity. Th2 cytokines drive the system toward immune tolerance rather than toward defense from microbial infections. Accumulating evidence suggests that Th1 responses in newborns are compromised at several steps including deficient production of Th1 type cytokines by neonatal CD4+ T-cells and hyporesponsiveness of neonatal macrophages to stimulation by IFN-γ. These deficiencies contribute to the apparently weak cellular immunity in newborns biased towards a Th2 type response [102].

Since the original Th1 and Th2 description, a differentiated T-cell population, Th17 cells, has been shown to have a pathogenic role in allergic, autoimmune and other chronic inflammatory diseases [103, 104]. Th17 cells have also been shown to play a protective role in immunity to infection, where they take on Th1-like effector functions to promote pathogen clearance by enhancing neutrophil recruitment to sites of infection and activating macrophages [103–105]. While the role of Th17 cells early in life remains unclear, cord blood mononuclear cells have a limited capacity to produce IL-17 [106]. In very premature infants with a history of serious bloodstream infections (BSI), we found lower serum IL-17 levels than their counterparts who never developed BSI.

The fetus occupies a unique immunologic position where the Th2 phenotype predominates in utero and transitions to a more Th1 functionality following birth. It is generally considered to be a core process of evolutionary significance where both mother and fetus maintain a high level of immunologic suppression to enable continuation of pregnancy.

Antigen-Specific Responses

The response of T-cells to specific antigens can also be assessed in terms of proliferation or cytokine production. These responses usually require previous exposure to the corresponding antigen, and are generally not detected at birth or from cord blood unless there was an intrauterine exposure. Neonatal T-cells do, however, respond well to certain antigens such as tetanus/diphtheria toxoids, influenza, and mycobacterial antigens [87]. In response to superantigens, cord blood T-cells produce lesser amounts of IL-2 [107]. However, after stimulation, the percentage of $V\beta_2$+ T-cells (which determine potential reactivity to superantigens) and the number of memory T-cells increase significantly just like adults [107]. But unlike adult T-cells, cord blood T-cells are unable to respond if restimulated with the superantigen. This tolerance induction in cord blood T-cells may again be due to the underlying immunologic naiveté [107, 108].

108.4.2.5 Other Subgroups

Cytotoxic T-Lymphocytes (CTLs)

CTLs are important in host defense against intracellular infections, in allograft rejection and tumor cell surveillance [109]. CTLs utilize two well-established mechanisms for cell lysis, one involving release of extracellular mediators (such as the pore-forming perforin/granzyme system), and a second fas/fas ligand dependent pathway that leads to target cell apoptosis [110].

CTL cytotoxicity is evident by 18 weeks of gestation, but is far less efficient that adult cells even at term (<20 percent of adult CTL activity) [111]. Perforin expression in neonatal CTLs is about 30 percent of adult levels. CD28, which is a T-cell activation marker, is also expressed to significantly lower levels [90]. Similar results are also observed in other assays; neonatal cells showed only 33 percent of lectin/mitogen-dependent cytotoxicity of adult cells [112]. Circulating inhibitors such as a-fetoprotein and prostaglandins may also lead to lower CTL activity in neonates [112].

γδT-Cells

The γδ T-cells represent a distinct functional subset, with a majority lacking surface expression of both CD4 and CD8 [113]. These cells are detectable in the fetal thymus and liver at 6–8 weeks of gestation, and comprise nearly 10% of the peripheral blood T-cells at 16 weeks [114]. Subsequently, the numbers decline gradually to reach about 3% at term [115]. These cells are present mainly on skin and mucosal surfaces [113]. Although the exact functions of these T-cells are not well understood, they can lyse target cells with the perforin/granzyme system like the cytotoxic T-cells, and can secrete cytokines like interferon (IFN)-γ and tumor necrosis factor (TNF)-α upon activation. The cytotoxicity of neonatal γδ T-cells is significantly less than adults [116].

Fetal γδ T-cells have a more diverse repertoire but a more limited junctional diversity than adults. This diversity is retained throughout the first year of life, and then decreases gradually over the first decade of life [116]. Overall, however, γδ T-cells have a relatively restricted repertoire in comparison to the αβ T- or B-cells [117].

T-Regulatory Cells

T-regulatory cells (Tregs) downregulate T-cell responses to both foreign and self antigens, thereby playing an important role in balancing Th1/Th2 effector lineages [118]. Treg cells, including both natural $CD4^+$ $CD25^+$ Tregs as well as the IL-10-producing Tregs, express the forkhead/winged-helix family transcriptional repressor-p3 (Foxp3) [119], a commonly-used but not entirely specific marker for Tregs [120]. Although Tregs have been detected in cord blood, current knowledge about Treg function early in life is limited and thus their relevance to immune defense of the newborn is uncertain.

108.4.3 B-Lymphocytes

108.4.3.1 Development

B-cell progenitors, pro-B-cells, are derived from pluripotent hematopoietic cells in the bone marrow [121]. The first

recognizable B-cell progenitor, the large pre-B-cell, is characterized by the presence of cytoplasmic μ heavy chains [121]. Immature B-cells undergo a selection process analogous to T-cells to eliminate self-identifying clones (clonal selection, clonal deletion), although there may also be other mechanisms to maintain self-tolerance [122]. Once B-cells begin to express surface IgM (sIgM), they are ready to leave the bone marrow to enter the peripheral circulation [123].

Pre-B-cells can be identified in the fetal liver as early as 7 weeks of gestation, and in the marrow by 12 weeks. sIgM+ B-cells are found in the fetal liver by 9 weeks and in the bone marrow, peripheral blood, and spleen by 12 weeks. B-cells with sIgA, sIgG, and sIgD isotypes appear between 10 and 12 weeks. There is also increased traffic to the lymphoid tissues, and by 22 weeks, the proportion of B-cells in the spleen, peripheral blood, and bone marrow is similar to that in adults. By 30 weeks, there are no detectable pre-B-cells in the fetal liver, and bone marrow becomes the exclusive site for B-cell maturation. Plasma cells are not generally found until 20 weeks' gestation. IgM/IgD+ B-cells populate the lymph nodes by 16–17 weeks' gestation and the spleen by 16–21 weeks. In fetal lymph nodes, primary nodules develop around the follicular dendritic cells by 17 weeks' gestation [124]. Considering the extreme degree of premature birth and survival in this era even below 24 weeks, it is perhaps no surprise that effective production of antibody for opsonization and enhanced cytotoxicity is limited.

108.4.3.2 Immunoglobulin Repertoire

Receptor diversity in the antigen binding site originates from DNA recombination involving various V, D, or J gene segments giving rise to a large number of V(D)J permutations [80]. Additional receptor diversity is generated by imprecise gene segment joins, additional nucleotides added to the splice junction of the VDJ joins by the enzyme terminal deoxynucleotidyl transferase (TdT), and somatic mutations (for B-cells only, not T-cells). Thereafter, these VDJ or VJ (light chains) units join to their respective constant region gene segments [125].

In the fetus and neonate, the Ig repertoire is relatively restricted. During early and mid-gestation, certain heavy chain V gene segments are preferentially expressed. Early in fetal life, the most J_H proximal V_H gene segments are preferentially utilized, and consequently, the CDR3 region of the rearranged VDJ gene segment is shorter than that in adults. This leads to relatively limited junctional diversity, but this altered architecture of the antigen-binding site may also allow greater polyspecificity of antigen binding (at a cost of lower antibody affinity) [126]. The utilization of V_H gene families spreads out more evenly with increasing gestation. However, even at term, cord blood B-cells have a higher usage of V_H genes of the V_H 1 and V_H 5 family with decreased V_H 3 use compared with adult B-cells [127]. In general, the antibody response in neonates is of low affinity, and restricted to the IgM isotype. The somatic mutation of the heavy and light Ig variable region genes and the selection of higher affinity antibody-producing B-cells is limited at birth but increases very slowly after 10 days of age [128]. It is significant that the maturation of antigen presenting cells and functional limitations of B-cells reduces the capacity of effective defense mechanisms during the period of novel exposure to nutrient derived antigens and gut flora yet eventually acquires the ability to accurately discern self from diet.

108.4.3.3 Circulating B-Cells

At birth, the proportion of B-cells is similar to that of adults, but the absolute number of B-cells is significantly higher [129]. The number peaks at about 3–4 months of age, and then declines to adult levels by 6–7 years of age [130]. Preterm infants have comparable B-cell numbers to the term infants [131]. However, the number is smaller in growth-retarded infants. Unlike adults, most B-cells in cord blood express activation markers (CD25, CD23, transferrin receptor) [132].

108.4.3.4 Function

Immunoglobulin Production

The fetus and the neonate are capable, although at a lower intensity than adults, of mounting antigen-specific antibody responses. The presence of allergen-specific IgEs in cord blood, anti-tetanus IgM in cord sera of newborns whose mothers were vaccinated during pregnancy, and reactivity to *Ascaris* antigens in the event of maternal infestation are some examples [133, 134].

However, this response remains immature [124]. They may be unable to respond to all the antigens in a vaccine, and often have delayed isotype switch [135]. It appears that the interval from birth is a more important determinant of antibody response than the gestational age. Both preterm and term infants immunized with diphtheria toxoid at 0–10 days of age had poorer responses than similarly immunized adults, but the response was better when vaccination was deferred until 1–2 months of age [135]. With certain antigens like hepatitis B, however, premature infants may show a relatively poorer early response than their term counterparts, although these deficiencies correct during later infancy [136]. Collectively, these observations suggest that many of the maturational signals needed to achieve a full capacity of host defenses arise from evolutionary appropriate cues received from the neonatal environment forcing the irrevocable expansion of a wide diversity of immune capacities. Thus, the newborn is effectively, not solely limited by its ontological immaturity of cellular and humoral capacities.

Serum Immunoglobulin Levels

Serum Ig levels remain very low until 18–20 weeks' gestation. Most of the newborn's serum immunoglobulins are derived from active transplacental transfer of maternal IgG (particularly IgG1 and IgG3) during the third trimester [137]. In the full-term neonate, serum IgG levels are equal or even higher than maternal serum IgG levels, but in the preterm, who missed these maternal antibodies, the levels are lower.

Hobbs and Davis found that nearly all infants born before 32 weeks' gestation had serum IgG levels less than 400 mg/dL at birth (compared with term infants who had serum levels around 1000 mg/dL) [138]. The levels fall after birth (through normal catabolism) to a nadir of 300–500 mg/dL between 3 and 5 months of age, when the infant starts producing increasing amounts of his/her own. Because of starting at a lower level, this nadir may be much lower, and earlier, in preterm infants [139]. Cord blood Ig levels also tend to be lower in growth retarded neonates [140].

The serum levels of IgA, IgM and IgE are very low even in term infants, since these do not cross the placenta. However, when faced with an intrauterine infection, the fetus is definitely capable of producing appreciable amounts of IgM [141]. For many years it was believed that augmenting the neonatal repertoire with exogenous pooled IgG could augment immune functions. Unfortunately, data proving efficacy of this approach for prevention or intervention for bacterial infections has not proven compelling; nevertheless, use of high titer Ig for mitigating perinatal transmission of hepatitis B is well established.

Taken together, it is clear that an improved understanding of these IgG relationships may some day be exploited to enhance bacterial opsonization.

108.4.3.5 Other Subgroups

CD5 Expressing B-cells

B-cells with surface expression of CD5, which is a T-cell antigen, may represent a functionally and ontogenically distinct subset. Some believe that CD5 positivity defines a so-called B-1 subset of cells, distinct from the conventional adult B2 population by virtue of their earlier appearance in ontogeny, capacity for bone marrow-independent self-renewal, and constitutively expressing signal transducer and activator of transcription-3 (STAT3) [142]. B-1 cells express the B-cell-lineage antigens CD19 and CD45R, although CD45R is present at lower levels on B-1 cells than on B-2 cells [143–145]. B-1 cells in the peritoneal and pleural cavities can be identified by their unusual CD11b+ sIgMhi sIgDlow phenotype and can be further subdivided on the basis of differential expression of the cell-surface antigen CD5, into CD5+ CD11b+ $sIgM^{hi}$ $sIgD^{low}$ B-1a cells and CD5- CD11b+ $sIgM^{hi}$ $sIgD^{low}$ B-1b cells [146].

These cells are the predominant B-cell type during fetal life, and have a distinctive anatomic localization in the fetal spleen and the peritoneal cavity. They appear in the spleen at 15 weeks, and are seen in the lymph node primary follicles at about 17 weeks of gestation [147]. In adults, CD5 expression can be found on about 25–35% of total B-cells and 1–7% of all the peripheral blood mononuclear cells. In contrast, CD5+ B-cells represent approximately 90% of the total cord blood B-cells. This number decreases to 75–80% during infancy, and reaches adult levels only by late adolescence [147].

The exact function of B-1 cells in the fetus is still unclear. The functional characteristics of B-1 cells such as the unique localization, broad polyspecificities, and a restricted Ig repertoire have been considered to indicate a role of these cells in the innate, rather than in adaptive immunity [148]. Unlike follicular B-2 cells that respond to protein antigens, and with T-cell help, undergo immunoglobulin heavy chain class-switching and affinity maturation, B-1 cells respond mainly to T-cell-independent immunogens that include carbohydrate antigens [143–145].

Recent observations indicate that the two types of B-1 cell, B-1a and B-1b, show functional differences during the immune response. B-1a cells spontaneously secrete IgM, which provides a first line of defense against certain encapsulated bacteria, such as *Streptococcus pneumoniae*, whereas antibody production by B-1b cells is induced and has a role in the ultimate clearance of the pathogen and in providing long-term protection [146, 149, 150].

108.4.4 T- and B-Cell Interaction

T-cell signals are crucial for the proliferation, differentiation, and survival of B-cells, and include both antigen presentation and humoral signals (cytokines like IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and IFN-γ) [151]. Several pairs of other receptor-ligand molecules are also involved, including the CD40-CD40 ligand (CD40L) in B-cell immunoglobulin isotype switching, and others like B7/CD28, CD11a (LFA-1)/CD54 (ICAM-1), and CD58 (LFA-3)/CD2 in T- and B-cell activation [151]. Only about 30% of naïve neonatal T-cells express CD40L, compared to 80% of the naïve T-cells from adults [152]. However, once primed with mitogen(s) and IL-2, which converts naïve T- cells to the memory phenotype, neonatal T-cell CD40L expression is upregulated to adult levels [153]. The capacity of neonatal B-cells to differentiate into plasma cells and undergo isotype switching can be variable (*vide infra*), but in general, neonatal T-cells are less efficient at providing humoral and CD40-dependent activation signals [154, 155].

108.4.5 Natural Killer Cells

108.4.5.1 Development

NK cells share some T-cell markers, but are not affected in many natural/experimental disruptions of the T-cell system [156–158]. It is conceivable that the two lineages derive from a common progenitor [159, 160]. Morphologically, NK cells are large granular lymphocytes, and have characteristic surface markers including the CD56/neural cell adhesion molecule and CD16/$F_c\gamma$ receptor IIIa ($F_c\gamma$RIIIa), a low affinity IgG receptor. They also express CD2, LFA-1 and cytokine receptors such as IL-2$R_{\beta\gamma c}$, IL-12R, IFN-γR and IL-15R_α [161].

NK cells can be detected as early as 6 weeks of gestation, and the number then increases progressively until birth. In cord blood, 10–15% of all lymphocytes are NK cells, which is comparable to adult peripheral blood [159]. The phenotype of fetal NK cells, however, is different from that of adults' cells. Fifty to 80% of fetal NK cells express CD3γ, ε, λ, and σ proteins, unlike a much smaller number in term infants, or in adults where only CD3σ is expressed [159]. On the other hand, only 30–50% of the fetal NK cells express CD16 (compared with more than 90% of neonatal and adult NK cells). Similarly, CD56 and CD57 are expressed poorly on fetal or neonatal NK cells, compared to nearly 50 percent positivity in adult NK cells [159, 162].

108.4.5.2 Function

NK cells recognize viral-infected and tumor cells by the absence or decreased expression of MHC class I molecules on the cell surface [163]. Their MHC-unrestricted killing is mediated by perforin/granzyme apoptotic pathways [164]. The other mechanism of cytolysis is antibody-dependent cell-mediated cytotoxicity (ADCC), where target cell-bound IgG1 or IgG3 triggers the $F_c\gamma$RIIIa receptor on the NK cell [163, 165]. NK cells are also believed to play a key role in maintaining immunological tolerance at the maternal-fetal interface [166].

Fetal NK cells have a significantly lower cytolytic activity (including ADCC) against tumor cell target cell lines than adults, but it increases with gestational age parallel to increasing expression of CD56 and CD16 [153, 159]. However, even at term, the cytolytic activity is only 50–80% of adult levels [160].

NK cells should not be confused with the natural killer T-cells, a heterogeneous group of T-cells that express an alpha beta T-cell receptor along with some of the NK cell markers. Many of these cells recognize the non-polymorphic CD1d molecule, an antigen-presenting molecule that binds self- and foreign lipids and glycolipids. NK T-cells constitute only 0.2% of all peripheral blood T-cells. These cells play an important role in mucosal immunity and in the pathogenesis of inflammatory/allergic conditions; the role during fetal life remains unclear [167]. Imbalances in this system produced by chronic inflammation may be involved in the presentation of the acquired forms of hemophagocytic lymphohistiocytosis in the neonatal period.

108.5 The Mucosal Immune System

108.5.1 Peyer's Patches and Other Organized Lymphoid Tissue

Peyer's patch anlagen become identifiable in fetal ileum at 11 weeks as aggregates of HLA-DR^+, $CD4^+$ lymphoid cells [168, 169]. Major events in Peyer's patch development have been summarized in Fig. 108.3 and Table 108.1 [169–171]. At birth, the organized lymphoid compartment is naïve but structurally complete, and the predominant activity involves proliferative expansion (rather than primary lymphopoiesis) [171]. The number of PP increases from about 60 at birth to over 200 by 12–14 years [172].

In the vermiform appendix, the development of lymphoid structures lags behind the Peyer's patches [173]. Appendiceal

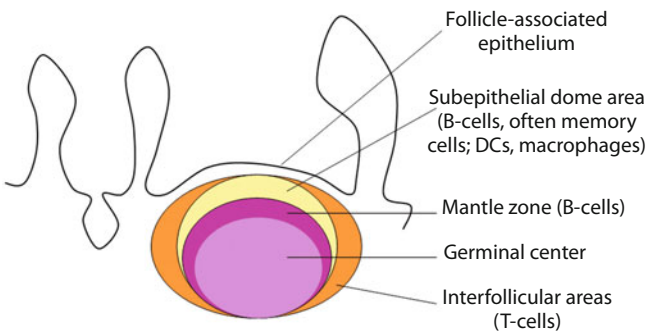

Fig. 108.3 Structure of Peyer's patch

Table 108.1 Development of Peyer's patches in the human fetus

11 wks gestation	PP anlagen with HLA-DR+ CD4+ lymphoid cells
16 wks gestation	Appearance of T- and B-cells First appearance of CD8+ cells* B-cell maturation with appearance of surface IgM and IgD
16–18 wks gestation	Appearance of CD5+ B-1 cells Surface IgA on B-cells
18–20 wks gestation	Appearance of PP zonation into B- and T-cell areas
24 wks gestation	PP are macroscopically identifiable
0–4 wks postnatal	Germinal center formation

* Fetal Peyer's patch T-cells are predominantly of the $CD4^+$ phenotype.

lymphoid follicles enlarge rapidly after birth following bacterial colonization and translocation [174]. The first IgA^+ plasma cells appear at 2 weeks after birth and then increase to adult levels at 4–5 months. To what extent full and appropriate capacity of this system contributes to the susceptibility of the preterm neonate to the development of necrotizing enterocolitis remains to be ascertained.

108.5.2 Lymphocytes in the Lamina Propria and Intra-Epithelial Compartments

Scattered B-cells are first observed in the lamina propria at 14 weeks gestation [169]. The fetal intestinal B-cell population consists of two distinct cell types. The first population of large, dividing, mature B-cells shares morphologic and phenotypic ($CD20^+IgM^+IgD^+$light $chain^+$) features with the thymic B-cells. These are large-sized cells with extensive cytoplasmic processes, which are in contact with adjacent T-cells. A second population of smaller pre-B-cells (IgM^+ light $chain^-$ $CD20^-$) has also been identified, suggesting the presence of local B-cell development [175]. As extrathymic T-cell development occurs in human fetal intestine (*vide infra*), it has been hypothesized that as in the thymus, the B-cells may play a role in the development and selection of the T-cells.

The B-cell population in the fetal intestine comprises IgM^+ and IgG^+ cells [176]. The fetal intestinal B-cell repertoire is similar to B-cells in circulation or other organs, but differs significantly from plasma cells in postnatal intestine [175]. After birth, the IgM^+ plasma cell population expands faster than IgG^+ cells, and at the same time microbial stimulation induces B-cells to undergo IgA class switch in both the lamina propria and organized lymphoid tissue [177]. IgA+ plasma cells are first seen in the lamina propria during the second postnatal week [178]. The number of IgA^+ cells in the mucosa reach adult levels at 2 years, although serum IgA concentrations reach adult levels only during the second decade [170].

Intestinal T-cells can be identified from 12–14 weeks of gestation [179]. Outside the organized lymphoid tissue, intestinal T-cells are distributed as intra-epithelial (IELs) and lamina propria lymphocytes (LPLs). The fetal gut has a small number of IELs (3–5 $CD3^+$ IEL/100 IECs compared to 6–27 cells/100 IECs in older children), which expand rapidly after birth (a 10-fold expansion of the αβ T-cells and a 2–3 fold increase in the γδ cells, vide infra) [170, 180]. In contrast, LPLs continue to expand during fetal period and have a density similar to the postnatal intestine by 19–27 weeks gestation [179].

Several early lineage T-cell populations can be seen in the fetal intestine, suggesting that T-cells may develop locally in an extrathymic pathway [169, 171, 179, 181]. These immature T-cell lineages are shown in Fig. 108.4. Whereas most immature LPLs differentiate rapidly after birth, the differentiation of IELs is slower and continues through infancy [182]. In addition to phenotypic changes, intestinal T-cells also continue to undergo functional maturation during infancy and childhood. The TCR β-chain repertoire is polyclonal during fetal period and infancy and only gradually becomes restricted to the oligoclonal pattern characteristic of adults. This restriction is likely due to expansion of a few dominant clones, which are specific for the commensal bacterial flora [182].

In the fetal intestine, about 10–30% of IELs express the γδ T-cell receptor [169]. Rodent studies suggest that γδ cells may regulate IEC function, display cytotoxic activity and may promote antimicrobial immunity [183, 184]. Similar to αβ T-cells, the fetal/neonatal γδ repertoire is also polyclonal [185].

108.5.3 Secretory Immunoglobulins

Secretory immunoglobulins, IgA and IgM, play an important role in mucosal defense. Secretory IgA (sIgA) can be detected in mucosal secretions as early as 1–8 weeks after birth [186–189]. sIgM, on the other hand, appears transiently during early infancy [188].

sIgA levels rise during the neonatal period to reach an initial peak (as measured in saliva) at 4–6 weeks. In premature infants, sIgA appears in secretions at a similar chronological age as in full-term infants, although sIgA concentrations may be lower. If chronological age is corrected for prematurity, sIgA concentrations then become similar to matched full-term infants [190, 191]. Salivary IgA levels continue to rise up to 18 months of age [191]. A transient nadir in sIgA has been inconsistently [189, 192] recorded at 3–6 months [188, 193].

Secreted immunoglobulins also change qualitatively during the first year. There is a switch from monomeric IgA to polymeric sIgA sometime during the first year, indicating maturation of the secretory immune system [194], or alternatively, increasing exposure to exogenous antigens [195]. The relative amounts of IgA subclasses in mucosal secretions also changes during infancy. At birth, sIgA1 is the dominant subclass but sIgA2 increases rapidly by 6 months of age [192].

Specific sIgA responses appear to be related more to the timing and quantum of the antigenic stimulus than to developmental factors during infancy. sIgA antibodies to *E. coli* somatic antigens appear in neonates within a few weeks after timed exposure and colonization [196]. The strength of the stimulus also has an effect: earlier, and stronger, specific sIgA responses are seen in neonates born in areas endemic for a pathogen compared to infants in the developed world [194, 197].

During the neonatal period, colostrum provides an important alternative source of sIgA [198]. Milk antibodies, amounting to about 0.5–1 g/day throughout lactation (comparable to the 2.5 g/day being produced by a 65 kg adult), are directed against antigens present in the environment shared by the mother-infant dyad [199]. The presence of enteromammary and bronchomammary pathways allow immune cells stimulated by antigens in the maternal intestine and bronchial mucosa to migrate to the mammary gland [198, 200]. Interestingly,

sIgA levels have been reported to be higher in colostrum and milk of mothers of preterm neonates [201].

Premature infants lack the intrinsic protective mechanisms of the adult intestinal mucosa that prevent sensitization against luminal constituents: a strong physical barrier, luminal enzymes that can alter ingested antigens, presence of regulatory T-cells, and the production of sIgA [202]. The risk of sensitization is further increased due to several developmental deficiencies within primary immune cells: (1) specific antibody responses in premature infants are abnormal due to reduced antigen affinity, increased polyreactivity, and autoreactivity [147, 203]; (2) the lengths of immunoglobulin heavy chain third complementarity determining regions (CDR3) are almost 3 amino acids shorter in the fetus/premature infant than adults [204]. This reduces the potential antibody diversity available to the fetus/preterm neonate by about 20^3 (= 8000) fold [204]. Moreover, antigen binding sites with short CDR3 regions, due to their tertiary structure, are more likely to bind to peptides such as allergens [205]; and (3) the short CDR3 regions of fetal $CD5^+$ B1 cells share characteristics with variable regions of IgE heavy chains [206, 207]. These observations have led to the hypothesis that B1 cells may contribute to the repertoire of allergen specific IgE^+ plasma cells, and that premature exposure of the immature intestinal B-cell repertoire to allergens may influence the risk of sensitization [207].

108.5.4 Intestinal Macrophages and Dendritic Cells

Macrophages first appear in the developing intestine at 11–12 weeks of gestation, increase rapidly during the 12–22 week period, and then continue to expand at a slower pace through early childhood [176, 208, 209]. These cells play a critical host defense role in being the first phagocytic cells of the innate immune system to encounter luminal bacteria that breach the epithelium and gain access to the lamina propria [60, 210]. Intestinal macrophages display avid phagocytic and bacteriocidal activity but are markedly attenuated in their inflammatory responses [60], a unique adaptive mechanism that prevents unnecessary inflammation in the gut mucosa despite the proximity to luminal bacteria. In sick and preterm neonates who are predisposed to bacterial translocation due to an abnormally permeable gut epithelial barrier, immaturity of the local adaptive immune system and low secretory IgA production [174, 211], intestinal macrophages assume even greater importance as a host defense system because of their ability to eliminate previously unknown bacteria through phagocytosis and intracellular killing. A breach of the gut mucosal barrier defense is met by tissue macrophages in the liver (Kupffer cells).

Intestinal macrophages are derived from circulating monocytes, which are recruited to the mucosa under the influence of various epithelial- and mesenchymal cell-derived chemoattractants [60, 210, 212]. Because neither intestinal macrophages nor their precursor monocytes have the ability to undergo clonal expansion [210], the only mechanism available for the development and maintenance of the gut macrophage pool is through the continuous recruitment and differentiation of blood monocytes. In adults, interleukin-8/CXC ligand 8 (IL-8/CXCL8) and transforming growth factor-beta (TGF-β) recruit macrophage precursors to the intestinal mucosa [210]. However, several lines of evidence indicate that IL-8 and TGF-β may not be important as macrophage chemoattractants in the fetal intestine. In the fetus, IL-8 is mainly comprised of a longer, less-potent 77-amino acid isoform (unlike the shorter 72-amino acid isomer in the adult) [213]. Similarly, TGF-β bioactivity is low in the early-/mid-gestation fetal intestine. Finally, macrophages appear in the fetal intestine at least a few weeks before lymphocytes or neutrophils [208, 209], suggesting that macrophage precursors are likely to be recruited to the early fetal intestine by chemoattractant(s) more specific for macrophage precursors than IL-8/CXCL8, which recruits both neutrophils and macrophage precursors [20, 210], or TGF-β, which mobilizes macrophage precursors as well as T lymphocytes [210, 214]. We have shown recently that epithelial cells in the fetal intestine produce chemerin (previously known as tazarotene-induced gene-2/TIG2 or retinoic acid receptor responder-2/RARRES2), to recruit macrophage precursors.

We have shown recently that unlike in the adult, intestinal macrophages in the midgestation fetus/premature infant are responsive to bacterial products and produce inflammatory cytokines. This inflammatory downregulation of fetal intestinal macrophages occurs under the influence of TGF-β, particularly the TGF-β_2 isoform. Further investigations are currently in progress to determine whether the incompletely-developed macrophage tolerance to bacterial products in the preterm intestine could predispose these infants to necrotizing enterocolitis.

There is very limited data on fetal/neonatal intestinal DCs [215]. $HLA\text{-}DR^+$ DC-like cells can be detected in both the lamina propria as well as the Peyer's patches after 14 weeks, but these cells may have some overlap with lamina propria macrophages. In rats and non-human primates, DCs have been noted in the fetal lamina propria as well in Peyer's patches [216]. The functional importance of these DCs is not clear.

108.6 Conclusions

It is apparent that the extraordinary complexity of the human immune system in general is amplified at the time of birth by the fetal stage of development, the postnatal age when examined, the ontological capacity of stem cells to respond to

maturational signals and each issue is compounded by the transition from a Th2 to a Th1 phenotype at the conclusion of pregnancy. All of these factors are occurring in tandem with a first inoculum of maternal bacteria as well as her repertoire of related antibodies followed by the acquisition of subsequent bacterial colonizers/modifiers of gut and skin flora (as influence by antibiotic exposure or ex utero environments) and nutrient-derived antigens that must all be distinguished from self to ensure an effective barrier defense and nutrient absorption. It is perhaps more surprising that this system works at all than the fact that there are limitations that increase vulnerability of neonates to infection. A fundamental and expanded understanding of these relationships will further the hope of identifying opportunities to augment immune defenses in ELGANs where bacterial invasiveness ranges from 30–50% of births.

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Congenital Immunodeficiencies

Alessandro Plebani and Gaetano Chirico

109.1 Introduction

The primary immunodeficiency diseases (PIDs) are a heterogeneous group of inherited disorders with defects in one or more components of the immune system. PIDs are classified into T lymphocyte, B lymphocyte, phagocytic cell, and complement deficiencies. This classification is simple and practical because clinical symptoms, mainly susceptibility to infections, vary according to the affected arm of the immune system. The prognosis of these disorders and their treatment depends on their early recognition and initiation of appropriate management.

Evaluation of immune function should be considered for children with clinical symptoms of a distinct immune disorder or with unusual, chronic, or recurrent infections, such as: one or more systemic bacterial infections (sepsis, meningitis); two or more serious bacterial infections (cellulitis, suppurative otitis media, pneumonia, lymphoadenitis) during the first year; serious infections occurring at unusual sites (liver, brain abscess, omphalitis); infections with opportunistic pathogens (*Aspergillus*, *Serratia marcescens*, *Pneumocystis jiroveci* (formerly *P. carinii*), *Cryptosporidium*, *Nocardia*); infections with common childhood pathogen but with unusual severity.

Clinical suspicion of a primary immunodeficiency disease should be raised in case of a history of consanguinity or a positive family history for a congenital immunodeficiency. Physical signs include an absence of lymphoid tissue or the presence of syndromic features. Severe or recurrent infections caused by encapsulated bacteria suggest a primary B-cell or complement defect, and they occur after the first 6-12 months of age, in parallel with the decrease of the maternally acquired IgG. On the contrary, severe viral (adenovirus, cytomegalovirus, respiratory syncytial virus), fungal or opportunistic infections during the first few months of life, are indicative of a primary T-cell defect.

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A complete differential blood count and assessment of immunoglobulin serum levels are the most cost-effective diagnostic screening tests. A normal neutrophil count excludes neutropenia, and normal platelet size or counts exclude the Wiskott-Aldrich syndrome. If the absolute lymphocyte count is normal, the patient is unlikely to have a severe T-cell defect because T-cells normally represent 70% of circulating lymphocytes and their absence results in severe lymphopenia. This is not the case for T^-B^+ severe combined immunodeficiencies, in which the presence of circulating B-cells results in a normal absolute lymphocyte count and cytofluorimetric analysis of B- and T-cell subsets reveals that virtually all lymphocytes belong to the B-cell lineage. Low immunoglobulin serum levels with normal T-cell counts indicates a diagnosis of a primary B-cell defect. Normal neutrophil, T- and B-cell counts should point to a functional defect of phagocytes. The genes responsible for many PIDs are known, which may lead to successful neonatal screening programs. In this chapter we will focus on primary T- and B-cell defects and related disorders.

109.2 Defects of the B Cell Compartment

109.2.1 Agammaglobulinemia and Common Variable Immunodeficiency

Agammaglobulinemia, X linked (XLA or Bruton's Agammaglobulinemia) or autosomal recessive agammaglobulinemia (ARA) represent the prototype of disorders of the B-cell compartment. Both disorders are characterized by absent/low immunoglobulin serum levels, defective antibody response to antigens, and absence of circulating B-cells in the presence of normal T-cell counts and function. Both disorders result from an early block of B-cell development, caused in XLA by mutations in the gene encoding Bruton's tyrosine kinase (Btk), which has a crucial role in early B-cell differentiation. XLA accounts for 70–90% of agammaglobulinemic patients. Five

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

distinct autosomal recessive genetic defects have been found to cause ARA, including mutations in the genes encoding the following: the μ heavy chain; Igα and Igβ signalling molecules; B-cell linker adaptor protein (BLNK); the surrogate light chain, λ5/14.1; and in the gene leucine-rich repeat-containing 8 (*LRRC8*). With the exception of *BLNK* and *LRRC8*, the other genes encode for components of the B-cell receptor complex, which is responsible for transducing the signals necessary for early B-cell development [1]. The onset of symptoms in agammaglobulinemia usually occurs early in infancy between 6 and 12 months as maternally acquired IgG decrease. Infections may start sooner in extremely preterm infants. A positive family history of primary immunodeficiencies is an indication for immunological investigation even in the absence of clinical signs. Bacterial infections are the most common clinical manifestations of agammaglobulinemia, usually by *Streptococcus pneumoniae*, *Haemophylus influenzae*, *S. aureus* and *Pseudomonas* species. Infections include otitis media, sinusitis, pneumonia, sepsis and meningitis. Less common bacterial species, such as *Salmonella* and *Campylobacter* account for gastrointestinal infections. Viral infections are usually handled normally with the exception of hepatitis viruses and enteroviruses [1]. Live attenuated vaccines should not be administered. Physical examination often reveals lymphoid hypoplasia with minimal or no tonsillar tissue and no palpable lymph nodes. Patients found to have low/absent immunoglobulin serum levels should have their B-cells evaluated by flow cytometry using monoclonal antibodies against CD19 or CD20. Normally, approximately 10% of circulating lymphocytes are B-cells. B-cells are absent (or <1%) in both XLA and ARA. However, hypogammaglobulinemia in the presence of normal B-cell counts suggests a diagnosis of common variable immunodeficiency (CVID). This distinction is important because children with hypogammaglobulinemia due to XLA/ARA or CVID can have different clinical problems, and the two conditions clearly have different inheritance patterns. Although CVID is considered a disorder of adults, it may also occur in infancy.

Because of the transient hypogammaglobulinemia of infancy, a definite diagnosis of CVID cannot be made early in life unless a defective antibody response to antigens is documented. Gene sequence analysis of the genes causing CVID (*ICOS*, inducible co-stimulator; *TACI*, transmembrane activator, calcium modulator, and cyclophilin ligand interactor; *BAFF-R*, B-cell activating factor of the TNF family receptor, *CD19*, *CD20* and *CD81*), is useful for providing a definitive early diagnosis. However, mutations of these genes account only for 10% of CVID patients [1, 2].

Immunoglobulin replacement therapy is the treatment of choice in agammaglobulinemic patients. Current protocols are based on intravenous immunoglobulins (IVIG) or subcutaneous immunoglobulins (SCIG). Several international studies have shown that maintaining pre-infusion IgG levels > 500 mg/dL results in a notable reduction in the number of infections. This protective IgG level is usually achieved by administration of 400 mg/kg every 3–4 weeks.

109.3 The Hyper IgM Syndrome (HIGM)

HIGM is a heterogeneous group of genetic disorders characterized by normal/elevated IgM associated with low IgG and IgA serum levels because of a defect in the immunoglobulin class switch recombination process. There are X linked and autosomal recessive forms of the disorder [3, 4]. The X-linked forms are caused by mutations of the *CD40 Ligand* (HIGM1) or *NEMO* (nuclear factor κB essential modulator) genes. The autosomal recessive forms are due to mutations of the *AID* (activation-induced cytidine deaminase, HIGM2), *UNG* (uracil DNA glycosylase), or of the *CD40* gene (HIGM3). Distinct clinical features are associated with different genetic defects. HIGM1 and HIGM3 are the most severe forms and their clinical presentation is similar to that observed in immunodeficiencies characterized by T-cell defects. In fact, CD40L is expressed on activated CD4 T-cells and CD40 on B and dendritic cells and defective expression of these molecules ultimately leads to a defect of T-cell priming. Most patients present in infancy with bacterial infections of the upper and lower respiratory tract and have a unique predisposition to *Pneumocystis jiroveci* pneumonia (PCP). These patients are also susceptible to infection with opportunistic pathogens, such as Cytomegalovirus, *Histoplasma capsulatum*, *Cryptococcus*, and *Cryptosporidium parvum*, which is associated with chronic diarrhea. Infection of the biliary tree with Cryptosporidium parvum, may predispose patients to sclerosing colangitis and to tumor of the liver, pancreas or biliary tree. Neutropenia is a common finding in HIGM1. The HIGM caused by the *NEMO* gene is easily recognized because of the presence of anhydrotic ectodermal dysplasia with sparse scalp hair, conical teeth and absent sweat glands.

The HIGM syndromes due to mutations of AID and UNG genes are considered primary B-cell defects. There is typically an increased susceptibility to bacterial infections, similar to agammaglobulinemic patients often with lymphoid hyperplasia.

In the case of low/absent levels of serum IgG and IgA with normal/elevated IgM, the diagnosis of HIGM1 is usually made by demonstrating an inability of activated CD4+ T-cells to express functional CD40L. HIGM2 is diagnosed by demonstrating B-cells that are unable to express the CD40 molecule. Diagnosis of HIGM caused by mutation of AID or UNG genes requires gene sequence analysis.

Except for CD40L and CD40 deficiency, for which stem cell transplantation is recommended [3], appropriate use of antibiotics to treat infections and the regular administration of intravenous immunoglobulins are the only effective treatments for these disorders.

Patients with CD40L and CD40 deficiency should be given co-trimoxazole as prophylaxis for *Pneumocystis jiroveci* pneumonia, advised to drink boiled water, and azithromycin prophylaxis may lessen the risk of *Cryptosporidium parvum* infections.

109.4 Defects of the T Cell Compartment

109.4.1 Severe Combined Immunodeficiencies (SCIDs)

SCIDs are a heterogeneous group of congenital disorders characterized by block of T-cell differentiation, variably associated with abnormal development of other lymphocyte lineage, such as B or natural killer (NK) cells [5]. The overall frequency of these disorders is estimated to be 1: 50,000–1:100,000 live births. SCIDs include a large number of disorders with X-linked or AR-inheritance, most of which are now molecularly defined. The clinical presentation is quite uniform, among the various SCIDs. Affected patients present within the first few months of life with infections, mainly of the respiratory and gastrointestinal tract. Oral candidiasis, persistent diarrhea with growth impairment and/or interstitial pneumonitis are the most frequent manifestations. These patients have increased susceptibility to infections caused by opportunistic organisms including *Candida albicans*, *Pneumocystis jiroveci* and *Aspergillus* species or by viruses (adenovirus, cytomegalovirus, respiratory syncytial virus). Live attenuated vaccines are another cause of severe clinical manifestations. BCG vaccination may lead to disseminated, often lethal infection; progressive central nervous system poliovirus infection can occur secondary to oral polio vaccination or exposure to a recently vaccinated individual.

Non-infectious clinical manifestations consist mainly of graft-versus-host disease (GVHD), presenting with a severe skin rash, caused by maternal engraftment or following transfusion with non-irradiated blood derivatives.

A diagnosis is possible at birth, with most affected infants having lymphopenia (less than 2000 lymphocytes/mm^3). Lymphocyte phenotyping shows a low number of T lymphocytes. B lymphocytes and NK cells may be present or absent depending on the type of SCID. If a high T-cell count is against a diagnosis of SCID, but there are typical clinical symptoms, a systematic search for the maternal origin of the circulating T-cells should be performed. Serum immunoglobulin concentrations are diminished or absent and no antibody response can be elicited after immunization. Four lymphocyte phenotypes are possible on the basis of the influence of the defective gene on T-cell, B-cell, and NK cell development. The $T^-B^+NK^+$ immunophenotype is due to CD3δ–, or CD3ε–, or CD3ζ, or interleukin-7 receptor α-chain deficiency, the $T^-B^+NK^-$ form includes the X-linked SCID which is due to γc deficiency, and the autosomal recessive forms due to JAK3 (Janus kinase 3), or CD45 deficiency, the $T^-B^-NK^+$ form is caused by RAG1, RAG2 (recombination-activating gene), or Artemis deficiency, the $T^-B^-NK^-$ form is caused by ADA (adenosine-deaminase) deficiency. A total lack of both lymphocytes and granulocytes, is suggestive of reticular dysgenesis which is due to mutations of *AK2* (adenylate kinase 2).

The natural course of SCIDs is severe, with most patients dying within the first years of life, unless properly treated. Supportive therapy, which may at best prolong survival, consists of intravenous immunoglobulins, aggressive treatment of infectious episodes, prophylactic co-trimoxazole (to prevent *Pneumocystis jiroveci* pneumonia), and irradiated blood products. Bone marrow transplantation (BMT), or other stem cell transplantation, often results in permanent cure, with a survival rate of 90% if an HLA-identical family donor is available. Excellent results (80% survival) have been obtained with HLA-matched unrelated donor and with aploidentical donor (75% survival). ADA-deficient SCID and X-linked SCID have been treated with somatic gene therapy; although serious adverse events occurred in the case of X-SCID. ADA-deficient SCID is also managed with regular injections of polyethylene glycol conjugate to the bovine-derived adenosine deaminase (PEG-ADA).

109.4.2 Combined Immunodeficiencies (CID)

CID is distinguished from SCID by the presence of low but not absent T-cell function. Similar to SCID, CID is a syndrome of various genetic defects. Clinical presentation may overlap with that of SCID patients, although sometimes infections develop slightly later. CID is an heterogeneous group of immune disorders among which the following are included.

109.4.2.1 MHC Class II Antigen Deficiency

MHC class II antigen deficiency is an autosomal recessive primary immunodeficiency caused by the absence of MHC class II expression on cells normally expressing HLA class II molecules [6]. This disorder is caused by mutations in several different genes, which code for a complex of regulatory factors controlling transcription of MHC II genes. MHC class II-deficient patients have a very low number of CD4 T-cells but normal or elevated numbers of CD8 T-cells. Lymphopenia is only moderate. Patients are hypogammaglobulinemic due to impaired antigen-specific responses caused by the absence of antigen-presenting molecules. These patients present, from early infancy, with an increased susceptibility to viral, bacterial, fungal, and protozoan infections, primarily of the respiratory and gastrointestinal tract. Curative treatment is BMT, although it has limited success in comparison with other types of T-cell immunodeficiencies.

109.4.2.2 Wiskott-Aldrich Syndrome

The Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder characterized by microplatelet thrombocytopenia, eczema, recurrent infections, and increased risk of autoimmunity and lymphoreticular neoplasia [7]. Thrombocytopenia and small platelet volume in a male should raise suspicion of the diagnosis. This disorder is caused by mutation of the *WASP* (WAS protein) gene. *WASP* is expressed in

all hematopoietic cells, including CD34+ stem cells. Mutation of this gene not only cause WAS, but also X-linked thrombocytopenia (XLT), a milder form of the disease characterized by microplatelet thrombocytopenia, but without the clinical findings associated with the classic WAS phenotype. The classic clinical symptoms (bleeding, infections and eczema) are usually not present simultaneously. The earliest manifestation, often present at birth, consists of petechiae and bruises. Additional early manifestations of thrombocytopenia include bloody diarrhea, and hemorrhage following circumcision. Eczema and recurrent bacterial infections usually develop during the first year of life; later, infections with microorganisms such as *P. jiroveci*, and the herpes viruses become more frequent. The severity of the immune deficiency can vary depending largely on the type of mutation and its effect on protein expression. Both T and B lymphocyte functions are affected. During infancy the number of circulating lymphocytes might be normal or moderately decreased. Consistent findings are low isohemagglutinins titers, and marked decreased response to polysaccharide antigens.

Intravenous immunoglobulin, appropriate treatment and control (use of killed vaccines) of infections and eczema, platelet transfusion for serious bleeding episodes are part of an appropriate supportive therapy. Bone marrow transplantation is the treatment of choice and may be curative.

109.5 Immunodeficiencies as Part of Complex Diseases

109.5.1 DiGeorge Syndrome (DGS)

DGS is one of the most common chromosomal disorders known (estimated prevalence of 1/4000–1/6000 persons). It is caused by developmental defects in the third pharyngeal pouch and fourth pharyngeal arch [8] and defects are found in the thymus, heart and parathyroid glands. Approximately 90% of patients with DGS are hemizygous for 22q11; in rare instances, patients are hemizygous for 10p13. DGS is characterized by the triad of clinical features: congenital heart defects, immunodeficiency secondary to thymic hypoplasia, and hypocalcemia secondary to parathyroid gland hypoplasia. However, it is now well recognized that the phenotypic features of DGS are much more variable and extensive than previously recognized and frequently overlapping with other disorders including velocardiofacial syndrome (VCFS) and conotruncal anomaly face syndrome (CTAFS), both of which are frequently associated with 22q deletions. The collective acronym CATCH22 syndrome (Cardiac defects, Abnormal facies, Thymic hypoplasia, Cleft palate, and Hypocalcemia resulting from 22q11 deletions) has been proposed for these differing presentations, but should probably be avoided when considering individual patients. Dysmorphic facial features of DGS include: hypertelorism, low-set, prominent ears, micrognathia, high arched palate, short philtrum of the upper lip. Cardiac defects, dysmorphic facial features and hypocalcemic seizures occurring during the neonatal period should raise the suspicion of DGS.

All children suspected of DGS should have an immunological work up. Most infant with DGS have a mild and transient immunodeficiency. Immunoglobulin serum levels are usually normal, T-cell production is usually moderately impaired and T-cell mitogen responses are often normal or near normal. These patients usually develop normal or near-normal immunologic function over time. Total thymic aplasia is present in less than 1% of cases. It occurs in complete DGS, when patients resemble SCID in their immunological phenotype and susceptibility to infections with low grade or opportunistic pathogens. Bone marrow and thymus transplantations have been performed in complete DGS.

109.5.2 Cartilage-Hair Hypoplasia (CHH)

CHH is a rare autosomal recessive disorder characterized by chondrodysplasia with growth failure, hypoplastic hair, defective immunity and erythrogenesis. CHH is caused by mutations in the *RMRP* (ribonuclease mitochondrial RNA-processing) [9]. The disease is prevalent among the Old Order Amish in the United States and in the Finnish population. Growth failure, has its onset prenatally, and is usually due to short limbs. All segments of the limbs are affected. The major radiological abnormalities are confined to the methaphyseal parts of the tubular bones, which are flared, scalloped, and irregularly sclerotic. The majority of the patients have sparse, fine, and silky hair. Hirschsprung's disease and predisposition to malignancies have been reported.

The defective cellular immunity is characterized by mild to moderate lymphopenia and impaired *in vitro* lymphocyte response to mitogens. Humoral immunity is usually intact. In a few patients a more severe immunological phenotype, similar to combined immunodeficiency (CID), has been reported. Deficient erythrocyte production presents usually as mild macrocytic anemia in early childhood, with spontaneous recovery before adulthood. Patients occasionally present with severe congenital hypoplastic anemia.

Hematopoietic cell transplantation has resulted in successful immune-reconstitution in CHH patients who present with CID, but will not affect the morphologic features or dwarfism.

109.5.3 Nijmegen Breakage Syndrome (NBS)

NBS is an autosomal recessive genetic disease belonging to a group of disorders often called chromosome instability syndromes, characterized by microcephaly, particular "bird-like" face, growth retardation, immunodeficiency and predisposition to cancer [10]. NBS is caused by mutation of the *NBS* gene, which encodes for a protein involved in the repair of DNA double-strand breaks. Suspicion of NBS should be

raised by intrauterine growth restriction, microcephaly and facial dysmorphism (sloping forehead, receding mandible, prominent mid face, upward slant of the palpebral fissures). Chromosomal rearrangements, typically involving chromosomes 7 and 14, and chromosomal hypersensitivity to X-irradiation are useful laboratory tests to support the clinical suspicion. The definitive diagnosis is through gene sequence analysis: the 657del5 mutation is present in approximately 90% of cases. Hypogammaglobulinemia is common. Mild to moderate lymphopenia is present with an impaired *in vitro* lymphocyte proliferative response to mitogens. The primary cause of death is cancer, with a median of age of cancer-related death of only 10 years. Patients also develop recurrent upper and lower respiratory tract infections and chronic lung disease is the second leading cause of death. Differential diagnosis should include ligase-4 deficiency, which shows overlapping clinical and laboratory findings with NBS. Ataxia telangiectasia (AT), overlaps NBS in a number of characteristics, including chromosomal instability, radiosensitivity, and cancer predisposition, but AT patients do not display the characteristic "bird-like" facial appearance, microcephaly and growth retardation of patients with NBS.

109.5.4 Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX)

IPEX is a rare genetic disorder of immune regulation caused by mutations in the *FOXP3* gene located in the centromeric region of the X chromosome [11]. The product of this gene is required for the development of CD4+CD25+ T regulatory cells. In the absence of T regulatory cells, activated CD4+ T-cells produce multi-organ damage resulting in autoimmune manifestations such as type I diabetes mellitus, severe enteropathy, hypothyroidism, and autoimmune skin disease resembling eczema, psoriasis, or atopic dermatitis. These clinical manifestations, often fatal, usually occur in early infancy and often appear sequentially, rather than simultaneously. Immunologic testing reveals little beyond variably increased IgE levels, eosinophilia, mild increase in CD4:CD8 ratio and an increase in T-cell activation markers. Autoantibodies are often absent early but develop gradually or acutely. Immunosuppressive drugs have been used with some success. Unfortunately, these drugs do not maintain long-term remission of symptoms, may be toxic and facilitate opportunistic infections. Hematopoietic stem cell transplantation may be considered for the most severe forms.

109.5.5 Autoimme Polyendocrinopathy Candidiasis Ectodermal Dystrophy Syndrome (APECED)

APECED, also known as APS-1 (autoimmune polyglandular syndrome type 1), is an autosomal recessive disorder due to mutation of the *AIRE* (autoimmune regulator) gene [11]. The product of this gene is crucial for the induction of central tolerance through the regulation of self-antigen gene expression in the antigen presenting cells of the thymic medulla. Thus, defects of *AIRE* lead to a defective selection of organ-specific T-cells, which are responsible for autoimmune manifestations. APECED is characterized by a set of three abnormal features: chronic mucocutaneous candidiasis, hypothyroidisms and adrenal insufficiency. At least two of these major components need to be present for diagnosis. Usually the first sign of the syndrome is mucocutaneous candidiasis, which may occur soon after birth, while other signs appear later. High serum antibody titers to components of the affected endocrine organs are characteristic. Current management is supportive.

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Inflammatory Mediators in Neonatal Asphyxia and Infection

Marietta Xanthou and Victoria Niklas

110.1 Introduction

Inflammatory mediators produced in response to hypoxic-ischemic (H-I) injury and infection in the newborn, include multi-potent cytokines and chemokines released by a variety of somatic and bone marrow-derived cells both locally, in the brain and systemically, in the circulation. Pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-18, interferon (IFN)-γ and tumor necrosis factor (TNF)-α, are small polypeptides secreted in response to cellular injury and inflammation. Cytokines trigger somatic and immune cell activation, differentiation and death after signaling through their cognate receptors. Chemokines, such as CCL2 and CXCL8, are chemotactic cytokines that induce cells of the innate and adaptive immune system to leave the blood stream and migrate to sites of injury in the central nervous system (CNS) and in the periphery [1]. Cytokines and chemokines are considered critical mediators of brain damage and clinical indicators of overwhelming sepsis in the newborns. We review the cellular origin and role of cytokines and chemokines in these diseases. We also discuss treatment modalities that may interrupt these inflammatory cascades. A better understanding of the role of cytokines and chemokines in response to H-I injury and infection is likely to improve the outcome and long-term prognosis of these diseases in the newborn period.

110.2 Inflammatory Mediators and White Matter Injury in the Newborn

Hypoxia, ischemia and infection result in acute inflammatory responses and significant brain injury of the newborn. Moreover, preterm and low birth weight (LBW) infants may be at increased vulnerability due to cerebrovascular immaturity. White matter injury in the brain is a major cause of neurodevelopmental impairment and long-term disability in premature LBW infants [2]. Indeed, CNS injury following H-I injury and infection, as well as, their interplay with inherent vascular and oligodendroglial vulnerability of the immature white matter result in significant white matter injury and permanent neurodevelopmental impairment and disability [3]. Periventricular leukomalacia is one important correlate of the neurologic injury with significant morbidity in infants born at less than 1500 g. Approximately 10% of these infants exhibit cerebral palsy and 50% exhibit cognitive and behavioral deficits [4].

Cytokines and chemokines are known to be primary modulators of white matter injury in the brain of the newborn. Multiple cell types in the brain, such as microglia, astrocytes and neurons, as well as migratory cells of the innate and adaptive immune system, participate in injury via the secretion of pro-inflammatory cytokines and chemokines. Microglial cells, which also function as antigen presenting cells in the CNS, are triggered to release IL-1, IL-6 and IL-18, as well as neurotoxic substances, including excitatory amino acids [5]. Astrocytes release transforming growth factor (TGF)-β, IL-6 and the chemokines CCL3 and CCL5 [5], whereas glial cells directly enhance neuronal injury [4]. These cells also express receptors for inflammatory cytokines and chemokines allowing autocrine up-regulation of inflammatory responses to these mediators.

Increases in pro-inflammatory cytokines and chemokines in the CNS also function to recruit inflammatory cells, including granulocytes and lymphocytes, which are not normal inhabitants of the CNS. Recruitment occurs via cytokine-mediated activation of cell-type specific adhesion receptors on vascular endothelium and through the secretion of chemokines. Chemokines, such as CCL2 facilitate the accumulation of monocytes, which upon entry into the CNS, differentiate into macrophages at sites of injury. CXCL8 attracts neutrophils and CCL28, is a chemo-attractant for T- and

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

B-cells. Moreover, inflammatory mediators and cells of the innate and adaptive immune system also access the CNS more freely across a disrupted blood brain barrier (BBB) thereby minimizing the requirement for receptor-mediated migration of effector cells and soluble mediators.

Direct evidence that pro-inflammatory cytokines and chemokines damage the developing brain comes mainly from experimental studies in animal models where the type of injury and its duration can be controlled. Injection of IL-1 leads to neuronal death and delayed myelination in neonatal rats [6]. Blocking of chemokines or their receptors inhibits neutrophil-mediated damage to the BBB in experimental models. In addition, chemokine receptor inhibitors prevent CXCL8 responses and reverse the lethal sequelae of sepsis in mice [4]. Finally, TNF-α induces cell death in mature human oligodendrocytes and, in the developing ones, is associated with increased apoptosis and reduced myelination [6]. Today, there is increasing evidence that inherited cytokine or chemokine polymorphisms influence the risk for pre- and perinatal brain damage [6].

110.3 Inflammation and Injury Caused by Hypoxia-Ischemia

Birth asphyxia is an important cause of H-I injury, perinatal mortality and lifelong neurodevelopmental morbidity, including cerebral palsy, learning disabilities and mental retardation in the newborn. Perinatal asphyxia occurs in approximately 4/1000 term births and is more frequent in preterm newborns [7]. During H-I injury, glutamate excitotoxicity, free radical and pro-inflammatory cytokine release results in injury to the brain [4]. The inflammatory response to tissue injury occurs in response to the excitotoxic cascade during the reperfusion period and contributes to the evolution of injury. Free radicals produced by activated inflammatory cells activate NF-κB in brain cells leading to pro-inflammatory cytokine and chemokine release [4]. Furthermore, according to the "danger model" theory, signals from stressed or damaged cells in the CNS may initiate an immune response during which inflammatory cytokines and chemokines are also produced further contributing to injury [4].

Pro-inflammatory cytokine blood levels of IL-6, IL-1β, IL-12 and TNF-α are increased in infants with H-I encephalopathy and are associated with abnormal neurodevelopmental outcomes [4]. We have found that IL-6 and IL-1β serum levels are increased compared to controls, but these levels do not differ between asphyxiated and infected neonates [4]. However, TNF-α levels are similar between infants with H-I insults and controls. Furthermore, cytokine increases correlate with the severity of the perinatal insults [4]. In support, Okazaki et al demonstrated that serum IL-6, CXCL8 and IL-10 levels in asphyxiated terms are higher than those of the controls, while IFN-γ is lower [8]. Premature infants with MRI-defined cerebral white matter injury have higher levels of IL-6, IL-10 and TNF-α in the cerebrospinal fluid (CSF) than in the serum. There is no correlation between CSF and serum cytokine levels [6]. Finally, increased levels of activin-A, a cytokine hardly studied in term newborns, are observed in premature infants with perinatal hypoxia [9].

Elevated serum chemokines are also found in response to H-I injury. CXCL8, known to be chemotactic for neutrophils, basophils and lymphocytes, is elevated in term newborns with abnormalities on MRI and adverse neurologic outcome following H-I injury [4]. We have found increased levels of CXCL8 and CXCL10 in the blood of neonates with perinatal asphyxia during the first 24 hrs of life [4]. Our studies have also demonstrated a dichotomy of reactivity of activated peripheral blood lymphocytes according to the inflammatory insult; during perinatal infection these cells express increased levels of CXCL8 mRNA whereas during asphyxia, they express increased levels of CCL2 mRNA [10].

110.4 Inflammation and Injury Caused by Perinatal Infection

Perinatal infections are known to play an important role in the pathogenesis of white matter injury in the brain as well as in the pathogenesis of preterm labor and the later development of chronic lung disease. In fact, intrauterine infection and inflammation complicate up to 35% of preterm deliveries compared with 10–15% of those infants delivering at term [11]. Early onset sepsis occurs in 15–19/1000 live births in infants born as less than 1500 g, however, the overall risk of infection among preterm infants is greatest after the first week of life [11].

The primary barrier to infection in the fetus is the uterine-placental barrier, which has innate immune properties, in addition to innate and adaptive immune responses provided by circulating cells of the maternal immune system. In addition, passive immunity from the active transport of immunoglobulin G (IgG) across the placenta imparts additional protection to infants born at term although coverage is less complete in the premature infant as transfer is incomplete until the last trimester. The innate immune functions of uterine and placental tissues derive from the expression of toll-like receptors (TLR) [12]. TLR are pattern recognition receptors (PRR) that initiate inflammatory responses after binding a variety of viral nucleic acids and bacterial products [13]. TLR signaling further elicits anti-microbial and inflammatory responses through production of free radicals, proteases and inflammatory mediators. Ureaplasma species are implicated in inflammation induced at the feto-maternal interface [14]. Chorioamnionitis is associated with increased levels of CXCL8 and IL-6, while microbial invasion of amniotic fluid is associated with increased levels of IL-6, CXCL8 and IL-18 [14]. The fetal inflammatory response syndrome correlates

with elevated cord blood IL-6 [14]. More than 30 years ago, Dammann and Leviton showed that a strong inflammatory challenge, such as intrauterine infection, elicits a fetal inflammatory response and contributes to brain damage in preterm infants. Since then, studies have revealed that inflammatory mediators, such as cytokines and chemokines, are important links between infection and brain damage [15].

All aspects of innate and adaptive immune function after birth are immature and are further compromised in the prematurely born infant. Innate immune responses are initiated via the activation of complement, the release of anti-microbial peptides by neutrophils, paneth cells and macrophages and the engulfment of bacteria by phagocytes. Activation of the adaptive immune system occurs following antigen processing and presentation by antigen presenting cells, such as dendritic cells. Complement proteins, Ig and anti-microbial peptides promote direct cell killing following the release of anti-microbial peptides and enzymes, as well as phagocytosis through opsonization by Ig. Paneth cells, at the base of the crypts in the intestine and in other mucosal sites produce a variety of antibacterial substances including defensins and cathelicidins [16, 17]. These are small cationic peptides with broad-spectrum antimicrobial action, released in response to bacteria or to components of bacterial cell walls [18]. They are found at significantly lower levels in the intestine during fetal life when compared with the term newborn and adult [19]. Thus, the decreased production of defensins by paneth cells may predispose the fetus or infants born prematurely to bacterial overgrowth and overwhelming infection.

The production of granulocytes in the bone marrow and their progenitors is limited in the term and premature infant and their function is impaired potentially contributing to bacterial overgrowth and invasion. Likewise, the inflammatory cascade induced by bacteria may include a reduced oxidative burst in the premature infant due to low levels of NADPH, thereby reducing the function of the innate immune system [20]. Regarding adaptive immunity, although considerable development of T- and B-cells occurs during fetal life, complete maturation of the immune system occurs after birth. The production of antibodies by B-cells may be delayed and lower levels of Ig are detected despite the active transport of maternal IgG. Moreover, antigen uptake, degradation, and presentation by antigen presenting cells, a vital step in the initiation of an adaptive immune response, is less efficient in the newborn, hence, the ability of the adaptive immune system to detect and respond to pathogenic organisms is impaired. T-cells in the newborn are also less responsive to antigenic stimulation and have a decreased proliferative response to a variety of mitogenic stimuli [21]. Finally, circulating and epithelial-associated levels of IgA and IgG are also reduced resulting in greater susceptibility of the newborn to infections and across mucosal surfaces [22].

Regarding cytokines and chemokines in neonatal inflammation, increased levels of IL-6, CXCL8 and TNF-α are observed in newborns with early-onset sepsis [6]. Elevated levels of IL-6, IL-12p70, IL-10 and TNF-α levels are higher in LBW infants compared to controls, but these levels decrease after 24 hours with late-onset sepsis [23]. Premature newborns produce increased levels of pro-inflammatory cytokines, such as IL-2, IL-6, IFN-γ, TNF-α, and anti-inflammatory cytokines, such as IL-4 and IL-10 during infection compared to non-infected controls [23]. Yerkovich et al recently showed that IL-6, IL-10, TNF-α and IFN-γ release, following *in vitro* lipopolysaccharide (LPS) stimulation of cord blood cells, is equal or greater than that of adults [24]. The chemokine CXCL8 has been extensively studied and considered as a serum marker of neonatal sepsis, particularly in the early-phases of disease [4]. CXCL8, CXCL10, CCL9, CCL2, CXCL1 are all increased in neonates with sepsis, but their levels decrease after 24 hours whereas CCL5 is similar to non-infected controls [23]. Interestingly, Tatad et al proposed that the type of infectious agent leads either to increased inflammation or defective immunity. For example a lower level of IL-6 in response to *Staphylococcus epidermidis* infection may indicate a basis for vulnerability to infection whereas a high CXCL8 response to pathogenic *Escherichia coli* in preterm newborns is consistent with uncontrolled inflammation [25]. Taken together these results suggest that it is not only the absolute level of cytokines, but also the balance for pro-inflammatory cytokines and their counter-regulatory anti-inflammatory counterparts that may be important in disease pathogenesis.

The pathophysiology of an infectious disease in the fetus and newborn may be exacerbated by the inflammatory response to the pathogen rather than by virulence characteristics of the pathogen. In fact, excessive secretion of pro-inflammatory cytokines and chemokines may be more damaging to the host than the pathogen itself. Certain studies indicate that neonates have an increased inflammatory response compared to adults [24, 26]. Our findings also showed that LPS-induced TLR4, CD14 expression and CXCL8 release by neonatal peripheral blood leukocytes were significantly increased compared to adults [27]. Thus, quite often an overwhelming systemic inflammatory response may be generated during early or late -onset sepsis, meningitis or necrotizing enterocolitis, resulting in multi-organ failure, brain injury or death. Infants with infections are more likely to have brain damage [11].

Moreover, as the susceptibility of the newborn infant to infection is well-known, it is not unreasonable to propose that multiple defects in both innate and adaptive immunity contribute to further dysregulation of cytokine and chemokine secretion and account for significant "bystander injury" in organs such as the brain.

It is also important to note that physiologic responses to overwhelming bacteremia and sepsis may lead to microcirculatory dysfunction, shock and cerebral hypo-perfusion, resulting in pathways that overlap with primary H-I injury in the brain (Fig. 110.1). Hence, determining the primary root

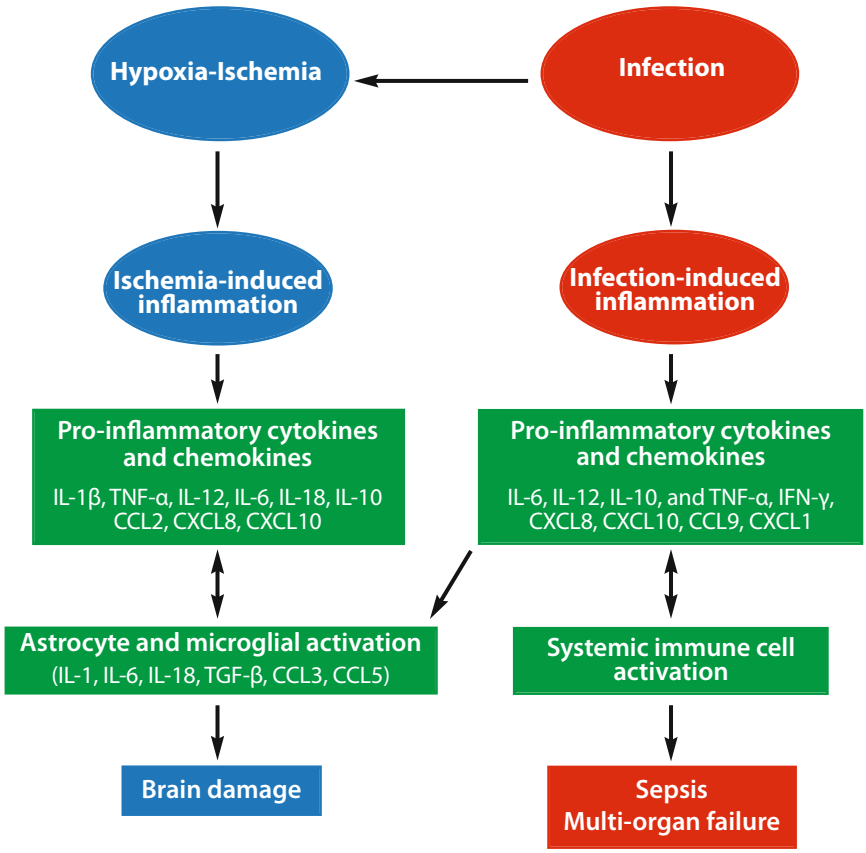

Fig. 110.1 H-I insults and infection lead to inflammation mediated by pro-inflammatory cytokines and chemokines produced by cells resident in the brain as well as by migratory bone marrow-derived immune cells. These mediators may either enter the brain and lead to white matter injury or cause systemic immune cell activation resulting in sepsis and multi-organ failure. Overwhelming infection can lead to H-I

of cause and effect of white matter injury in the brain during sepsis and shock may be difficult.

110.5 Cytokines and Chemokines as Therapeutic Targets in H-I Injury and Infection

Levels and ratios of pro-inflammatory cytokines and chemokines may provide the clinician with valuable diagnostic and prognostic information regarding inflammation induced by asphyxia and/or infection; however validation of these markers as prognostic indicators or as targets for treatment still needs to be documented. As yet, no definitive pattern or level of biomarker expression has been found to be predictive of disease severity or outcome, although certain cytokines and chemokines have been proposed [28].

Therapeutic interventions that either enhance the production or activity of anti-inflammatory cytokines or inhibit the production or activity of pro-inflammatory cytokines have been used quite successfully in a variety of animal disease models and in management of autoimmune and inflammatory diseases in humans such as in rheumatoid arthritis [29] and in inflammatory bowel disease [30]. However, clinical studies directed at modulating cytokine or chemokine function in newborn infants following H-I injury or infection with the goal of preventing overwhelming sepsis and minimizing white matter injury in the brain along with improving long-term outcome, are promising areas of research. Overall, targeting specific combinations of cytokines, chemokines and/or their receptors on brain and immune cells may inhibit excessive inflammation and injury, while sparing beneficial responses and protective immunity to infections.

110.6 Conclusions

Although neonates exhibit impaired expression of certain inflammatory cytokines and chemokines, such as IFN-γ, IL-12 and CCL5, the majority of inflammatory mediators are increased during infection and asphyxia to adult or more than adult levels. This could reflect the excessive inflammation often observed in neonates and, more so in premature infants, which can lead to septic shock and brain damage or the inability to mount an appropriate counter-regulatory anti-inflammatory response.

Understanding the mechanisms that contribute to the production of these pro-inflammatory cytokines and chemokines may lead to the identification of important therapeutic targets for brain damage and sepsis.

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Neonatal Malignancies

Franca Fossati-Bellani

111.1 Introduction

Neoplastic or tumor-like conditions are rare in the fetus and newborn, but pose important diagnostic and therapeutic problems for pediatricians and oncologists, as well as imposing enormous emotional burdens on parents and caregivers. They also present significant medical and ethical dilemmas.

By definition, neonatal neoplasms are those diagnosed during the first month of life. Congenital neoplasms are those diagnosed at birth. It is essential to define and distinguish tumor-like conditions (such as hamartomas, hemangiomas, lymphangiomas and melanocytic nevi) from benign or malignant tumors. Tumors that are benign at histology can still grow aggressively, acquiring a malignant behavior due to their site of onset and local invasiveness. They can cause death, as in the case of extensive lymphangiomas and teratomas of the newborn in the head, neck or mediastinum. On the other hand, histologically malignant tumors of the newborn, such as neuroblastoma stage 4S and neonatal fibrosarcoma, can regress spontaneously and take a benign course even without therapy. Finally, there are non-neoplastic conditions (i.e., abdominal masses) that can simulate the presence of a tumor, and that have to be differentiated by appropriate diagnostic procedures (Table 111.1). The vast majority of neonatal neoplasms are solid tumors of mesenchymal and embryonic origin, and they can be diagnosed before birth. Because they are so rare, the management of these tumors represents a major challenge for neonatologists, surgeons and oncologists: the knowledge gained from infants cannot be applied to the newborn because of the physiological immaturity of the latter's metabolism, hemopoietic and immune systems. Furthermore, current therapeutic protocols used in cooperative studies on childhood tumors fail to consider the peculiarities of the newborn.

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111.1.1 Incidence

Data on the incidence of neonatal malignancies are drawn from single-institution case studies or tumor registries, but there are no reliable general population based data available. Their incidence is estimated to be approximately 1 in every 12,500–27,500 live births. Neonatal malignancies account for 2% of all tumors affecting infants, children and adolescents. Half of neonatal tumors are found at birth, 20–30% during the first week and the remaining 20–30% within the first month of life. During the neonatal period, the incidence of a given tumor does not coincide with the related mortality rate because some tumors are rapidly lethal, while others cause death after the neonatal period, and yet others grow initially and then regress spontaneously, e.g., cystic neuroblastoma (NBL) and NBL in stage 4S. NBLs and teratomas (75% benign and 25% malignant) are the most frequent neonatal neoplastic conditions (accounting for 22% and 23%, respectively), followed by soft tissue sarcomas (8%), acute leukemias (6%), central nervous system (CNS) tumors (6%), benign and malignant liver tumors (6%), kidney tumors (7%), and retinoblastomas (5%). Males and females are generally affected equally, but retinoblastoma is more common in males, teratoma in females. Leukemias and CNS tumors are the most lethal in this age group.

111.1.2 Etiology

Neonatal congenital tumors constitute a unique model for studying the oncogenetics. The aging process and multiple environmental factors considered in adult studies are not applicable to the etiopathogenesis of tumors in children. Chemical and physical factors, and infections can influence the oncogenic processes involving the gametes, embryo and fetus. Genetic factors involved in the development of neoplasms, such as small mutations, the loss of heterozygosis and

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Table 111.1 Malignant (a), non malignant (b), tumor like conditions (c) of the newborn by location

Brain	a. Teratoma, PNET (medulloblastoma, pinealoblastoma, cerebral neuroblastoma), choroid plexus carcinoma, ependymoma, astrocytoma, atipical teratoid rhabdoid tumor, sarcoma, melanoma b. Teratoma*, meningioma*, hamartoma*, craniopharyngioma* c. Vascular malformation, hemorrage, hydrocephalus
Head and neck	a. Rhabdomyosarcoma (orbit, nasopharynx, middle ear), neuroblastoma (neck), retinoblastoma, teratoma, melanotic progonoma, Langherans cell histiocytosis b. Teratoma*, fibromatosis*, melanotic progonoma*, hamartoma, brachial cyst, lymphangioma* c. Cellulitis, nasopharyngeal brain heterotopia
Trunk	a. Neuroblastoma, germ cell, soft tissue sarcoma, Langherans cell histiocytosis b. Lymphangioma*, hamartoma*, teratoma*, cardiac rhabdomyoma c. Pulmonary myofibroblastic tumor, massive mesenchymal malformation of lung
Abdomen pelvis	a. Neuroblastoma, renal tumors, hepatoblastoma, germ cell tumors, soft tissue sarcoma, acute leukemia b. Teratoma, hepatic hamartoma, hemangioma, hemangioendothelioma, congenital mesoblastic nephroma* c. Polycystic kidney, hydronephrosis, urinary retention, gastrointestinal duplication, storage disease ovarian cyst, congenital viral infections
Skin and superficial soft tissue	a. Soft tissue sarcoma, neuroblastoma, acute leukemia, Langherans cell histiocytosis, melanoma b. Hemangioma, fibromatosis, giant naevus c. Infections

* Benign tumors potentially life threatening because of size and location or tendency towards malignant transformation.

changes in genomic imprinting, are more prominent in children than in adults. The correlation between malformations, genetic syndromes (hemihypertrophy, aniridia, Beckwith-Wiedemann syndrome) and tumors of childhood points to the conclusion that genetic factors are more significant than environmental ones in neonatal neoplasms.

111.1.3 Clinical Features and Diagnosis

Most tumors are clinically manifest as an abnormal mass located in the abdomen or head and neck, or any soft tissue site. They may be detected before birth. Advances in diagnostic procedures, e.g., ultrasound (US) and magnetic resonance imaging (MR), have facilitated the prenatal diagnosis of congenital neoplasms such as teratomas, abdominal or intrathoracic masses, with consequent implications for prenatal therapy and the choice of vaginal versus cesarean deliveries, and for the outcome of the fetus.

MR provides details of the site and the anatomical extent of a lesion and, in selected cases, it has been instrumental in strategic prenatal therapeutic procedures, such as fetal surgery or fetal rescue at the time of delivery. Computerized tomography (CT) is a diagnostic tool commonly used to diagnose tumors postnatally, but MR performs better in evaluating CNS neoplasms and spinal cord compression. Positron emission tomography (PET) has yet to be validated for use in diagnosing childhood tumors. Imaging data, combined with clinical findings, provide the basis for the histopathological diagnosis of neoplastic lesions that have been totally resected or biopsied. To characterize a neoplasm precisely, the morphological features derived from classic histology must be combined with immunohistochemistry and cytogenetic assessment. For instance, the value of the N-myc oncogene (a molecular marker of NBL) is significant in terms of prognosis and treatment.

111.1.4 Principles of Therapy

Interdisciplinary cooperation between all specialists involved at the various stages of the diagnostic and therapeutic process is vital.

111.1.4.1 Surgery

Surgical ablation is the therapeutic procedure of choice for most solid tumors (such as teratomas, Wilms' tumor and neuroblastoma).

Pediatric surgeons do not have to undertake aggressive surgery. The timing and strategy of surgical procedures have to consider the newborn's metabolic and physiologic needs, as well as the local extent of the tumor to enable complete excision, and the feasibility of delaying surgery until after shrinking the tumor volume by chemotherapy to avoid mutilating surgery or operations that would interfere with the child's growth or impair any vital functions. In pediatric oncology, the purpose of a multidisciplinary approach is to limit the damage associated with invasive therapies, especially in younger patients, without affecting adversely survival prospects.

111.1.4.2 Chemotherapy (CHT)

There are not enough pharmacologic studies of drug metabolism, clearance and toxicity to organs and functions to support the use of antitumor drugs in the newborn. Data on the pharmacology of antitumor chemotherapy in infants and young children have revealed severe neurotoxicity of vincristine, hepatotoxicity of actinomycin D, myelotoxicity of adriamycin and ototoxicity of cisplatin. Traditional cytotoxic drugs, administered at doses adjusted to the infant's weight enable drug clearance from the patient with fewer global toxic effects and a good therapeutic index. Using a patient's weight to guide dosage has been empirically adopted for the newborn. Drug dosage must be modified in the event of major side effects.

111.1.4.3 Radiation Therapy

The adverse effects of radiation therapy on infants and young children are now well known. They include irreversible damage to growth, the musculoskeletal system, and endocrine and cognitive functions, depending on the site and volume of the radiation fields. Furthermore, the risk of second tumors limits the use of RT in pediatric oncology and makes it contraindicated for the newborn.

111.1.4.4 Supportive Care

In pediatric oncology, good therapeutic results have been achieved (now reaching a 5-year survival rate of 70%) because of improved supportive care. Hydration, antibiotics, blood products, growth factors, antiemetic agents, and nutritional support are all particularly important for the newborn because of their inability to withstand invasive treatments and because of the severe risk of infection and metabolic complications in the short term. Central venous access is important [1–6].

111.2 Tumors

111.2.1 Neuroblastoma

NBL originates from neural crest cells and can occur along the sympathetic chain and in the adrenal medulla. The link identified at autopsy between infants dying of other causes and a high incidence of NBL in situ might be explained by this tumor's tendency for spontaneous regression. Most NBLs are associated with chromosomal anomalies, some of the most frequent being amplification of the oncogene N-myc, deletion of chromosome 1p, and aneuploidy. An amplified N-myc carries a poor prognosis [5-9].

111.2.1.1 Clinical Findings

The most common finding is an abdominal mass originating from the adrenal gland, but the primary tumor may be located in the mediastinum and neck, retroperitoneum or pelvis. Abdominal distension with or without respiratory insufficiency is a clinical finding that correlates with massive liver involvement from a small adrenal tumor. Secondary subcutaneous nodules and bone marrow invasion are also observed in this stage, defined as 4S. Other symptoms are due to the mass effect of the tumor, according to its location (respiratory insufficiency, or Horner syndrome if it is in the neck or mediastinal region) and/or to any metastasis.

111.2.1.2 Prenatal Diagnosis

When prenatal US identifies an abdominal mass, MR can differentiate between NBL and other tumors. In the prenatal phase, adrenal cystic lesions are interpreted as persistent or late-regressing neuroblastic nodules. They tend to regress spontaneously and they do not alter catecholamine metabolite levels, which can cause hypertension or maternal pre-eclampsia when abnormal.

111.2.1.3 Postnatal Diagnosis

Both CT and MR can distinguish between adrenal hemorrhages, renal masses and intra-abdominal sequestration (Figs. 111.1 and 111.2). Methyl iodobenzylguanidine (MIBG) scintigraphy can be used both to identify a neuroblastic tumor and to detect metastases. Only 70% of neonatal NBLs are MIBG-avid.

Histology is the final diagnostic tool, together with the previously-mentioned prognostic markers (N-myc, 1p deletion). If the liver is extensively affected (stage 4S), liver needle biopsy has been used instead of open abdominal surgery to reduce the risk of respiratory impairment and complications related to abdominal healing.

111.2.1.4 Staging

The INSS (Intergroup Neuroblastoma Staging System) criteria are used.

111.2.1.5 Therapy and Prognosis

The treatment program depends on the stage of the tumor and its biological characteristics. Children under one year or infants have a significantly better prognosis than older children. Currently cooperative groups recommend monitoring cystic

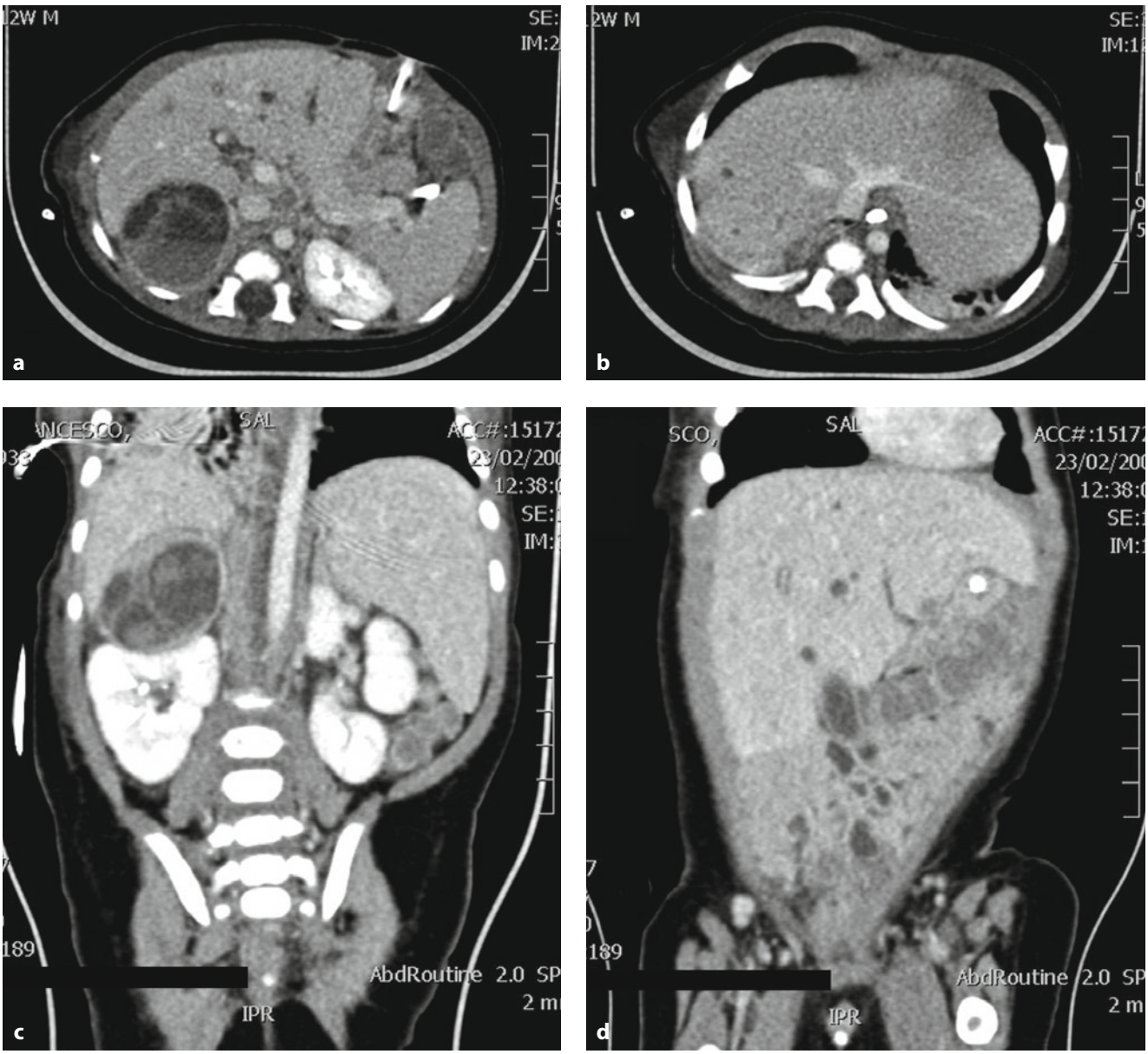

Fig. 111.1 Neuroblastoma 4S CT imaging. Right adrenal lesion, mostly cystic necrotic (**a**–**c**) with hematic level. Multiple metastatic little epatic lesions are present (**b**–**d**). (Courtesy Dr. Marcello Napolitano)

NBL during the first three months by US. Surgical resection is recommended when it neither regresses nor increases in size. Newborns in stages 1 and 2 have a good prognosis with surgery alone even when histology shows microscopic residues but no N-myc amplification in stage 2 cases. NBLs classified as stages 3 and 4 are prevalent in children over one year. Chemotherapy (CHT) is performed first to shrink the tumor, delaying surgery until later. For stage 3, the prognosis is excellent (about 90%), while for stage 4 it is 50%. If N-myc is amplified, high-dose intensive CHT with bone marrow rescue is recommended.

The prognosis is poor, however, especially when more than 10 pairs of the N-myc oncogene are identified. The drugs used are carboplatin, adriamycin, vincristine and topotecan. Newborns with stage 4S NBL have a good prognosis in 80% of cases without specific therapy. In cases with hepatic involvement and a risk of respiratory deficiency and/or vena cava compression, CHT can be implemented to accelerate tumor regression; surgery for the primary tumor is considered unnecessary. CHT and surgery are essential, however, in cases with biological markers indicating a poor prognosis [5–9].

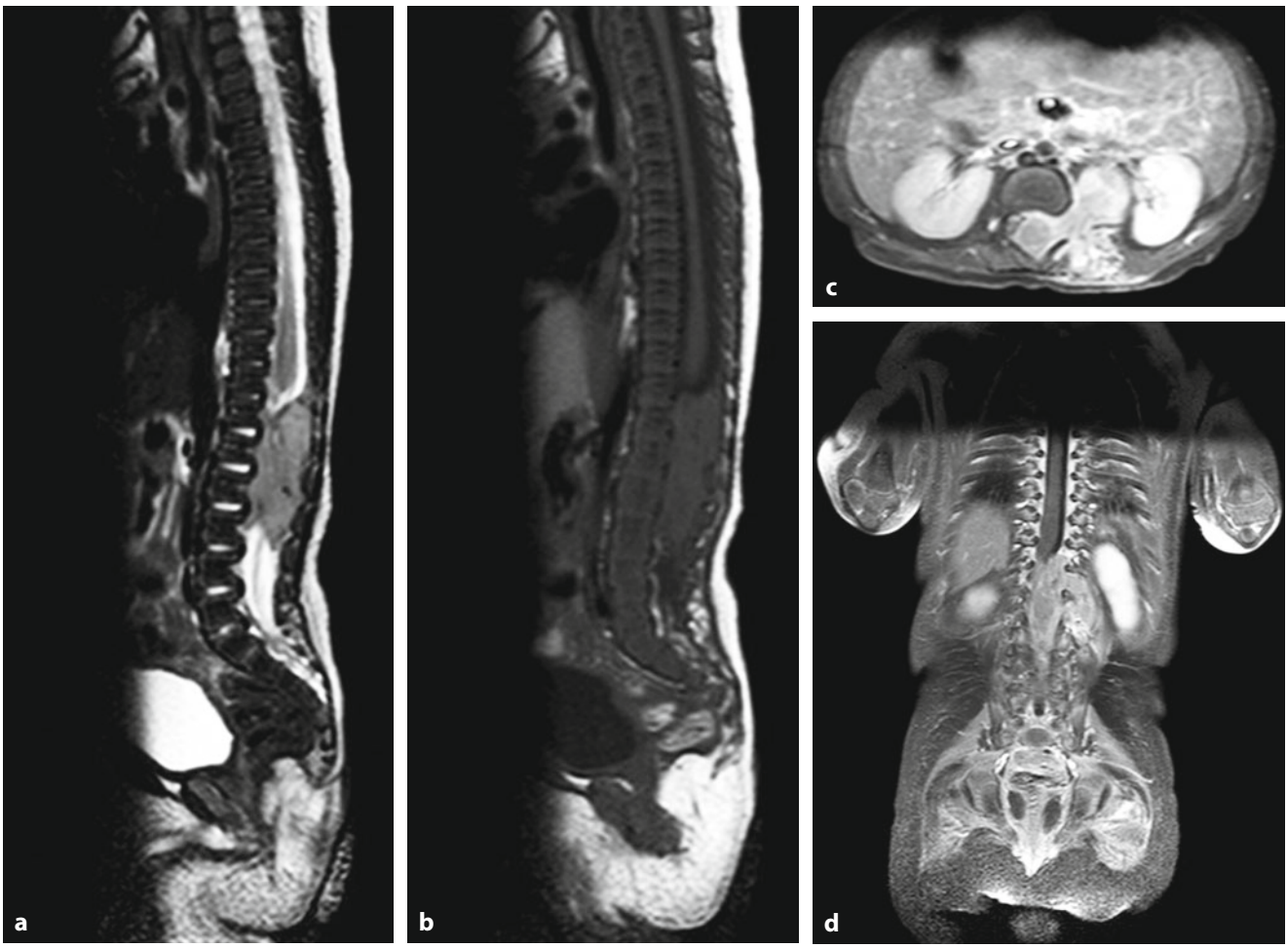

Fig. 111.2 Paravertebral left neuroblastoma with paraspinal and intrarachideal infiltration through conjugation foramina. MR imaging T2 W (**a**) and T1 W pre (**b**) and after (**c**, **d**) gadolinium. (Courtesy Dr. Cecilia Parazzini)

111.2.2 Germinal Tumors (GT) and Teratomas (TRT)

These are the most frequent benign and malignant perinatal tumors. More common in females, they are embryonic tumors that originate from primordial germ cells or germ layers (the ectoderm, mesoderm and endoderm). The World Health Organization (WHO) classification divides GT into 7 categories, but only TRT, yolk sac tumor and, more rarely, gonadoblastoma have been described in the perinatal period. More than 50% of TRTs appear at birth in the sacrococcygeal region, but they can occasionally be found in the head and neck, mediastinum, brain, retroperitoneum, and liver. TRTs are classified into three different groups, depending on their histology: (a) mature, consisting mainly of adult tissue; (b) immature, consisting of embryonic tissue; or (c) malignant, consisting of malignant embryonic tissue, which is almost always a yolk sac tumor.

The germinal tumor marker alpha-fetoprotein (AFP) levels are significantly abnormal in the malignant form and must be measured and monitored over time (considering as normal the levels during its normal regression in the first year of life). Approximately 10% of neonatal TRTs contain malignant cells, while 20% have immature components. Neonatal TRTs are often found in association with multiple or combined malformations of the genitourinary tract, rectum, anus and sacrococcygeal region, or of the face in the head and neck. Sacrococcygeal TRT may be post-sacral (external), pre-sacral (internal), or both (dumbbell). They are solid, cystic, or mixed masses containing varying proportions of all tissues, i.e., fat, muscle, bone, teeth, hair, immature neuroepithelial glandular tissue and glia. Prenatal assessment of the volume and site of the tumor is used to manage the delivery and to decide on perinatal measures such as intubation for airway obstruction in the case of cervical and mediastinal involvement. Treatment is surgical: open fetal surgery is indicated for large sacrococcygeal tumors when cardiovascular complications

and hydrops are present in mother. After delivery, the sacrum and coccyx may be removed. There must be accurate histopathology to evaluate the quality of the surgical excision, the entity and characteristics of the various cytological components, and the presence of immature or malignant tissue. Local relapses may occur in 10% of mature/immature tumors, especially if the surgical resection was marginal, or if the coccyx was not removed. The introduction of CHT with cisplatin plus etoposide in metastatic disease or for initially inoperable tumors has achieved complete regression of metastatic deposits and enabled radical surgery by reducing the tumor's initial volume. This strategy allowed a spectacular improvement in survival in both locally advanced and metastatic disease (the prognosis is good in 90% of cases of localized disease and 80% of metastatic cases). Adverse prognostic factors are even minimal presence of yolk sac tumor cells in mature and immature TRT and an incomplete resection [5, 6, 10–12].

111.2.3 Soft Tissue Sarcoma (STS)

Benign and malignant soft tissue tumors are the neoplasms most commonly seen in newborns after teratomas. They include conditions that differ in histology, molecular characteristics, biological behavior and clinical evolution. They also differ from the STS seen in adults. Malignancies include rhabdomyosarcoma (RMS), congenital fibrosarcoma, and adult-type sarcomas. These have to be differentiated from benign tumors and tumor-like conditions such as vascular lesions and fibromatosis [5, 6, 13, 14].

111.2.3.1 Rhabdomyosarcoma (RMS)

This is the most common malignant sarcoma to be found in the newborn, but only 14 cases out of 3217 (4%) were reported in the study of the American IRS (Intergroup Rhabdomyosarcoma Study). The embryonal histotype is most common, while the alveolar one is rare. RMS can occur anywhere in the body, e.g. in the head, neck, genitourinary area, and limbs. Diagnostic procedures include CT, MR, bone marrow aspiration and radiographs of the skeleton. Metastases may already be present at diagnosis. Symptoms may vary, e.g., acute urine retention (when the RMS is located in the pelvis) or cranial nerve palsy (when it affects the head-neck region), due to swelling in the area affected by the tumor. The botryoid variety includes grape-like neoformations, especially in the vulvovaginal area or mouth. Treatment depends on the stage of the tumor and is the same as for children under one year (or infants), as established in cooperative international studies. A combination of CHT and surgery is used, depending on the location and extent of the tumor, and whether it is amenable to surgical resection. Surgery may be the first step for small lesions, since it may be both diagnostic and therapeutic. Where surgery fails to completely remove the tumor, a second-look surgery is recommended after CHT. A combination of vincristine, actinomycin D and cyclophosphamide is the first therapeutic choice for inoperable or metastatic disease. The prognosis is less favorable in the newborn than in older children, especially in cases with the alveolar histotype.

111.2.3.2 Congenital Fibrosarcoma

This tumor occurs in the newborn and up to six months of age. It mainly affects the limbs, but the trunk, the sacrococcygeal and retroperitoneal regions, and the head and neck may also be involved (Fig. 111.3). The characteristic chromosomal translocation t(12:15) involving genes ETV6 and NTRK3 in the newborn does not appear in older children. For cases of locally extensive disease, primary CHT can shrink the tumor and delay the need for surgery.

111.2.3.3 Other Soft Tissue Sarcomas

The atypical teratoid rhabdoid tumor (ATRT) of soft tissues has the same chromosomal abnormalities, or INI gene mutations, as those observed in the more common ATRTs of the kidney, liver and brain of children more than one year old. It is rare in soft tissues and carries a poor prognosis. There are reports in the literature of other types of STS in the newborn, including peripheral primitive neuroectodermal tumor (pPNET), vascular tumor and undifferentiated sarcomas.

111.2.3.4 Congenital Fibromatosis

This is a tumor-like condition that needs to be distinguished from the above-mentioned malignant mesenchymal tumors. A common feature of congenital fibromatosis is the proliferation of elongated fibroblastic cells that tend to invade the surrounding tissues, but do not give rise to metastases.

a. Solitary or multicentric congenital myofibromatosis is more common in males and affects the head and neck, trunk and skin. It may regress spontaneously.
b. A sternomastoid tumor is generally connected with birth trauma. It must be differentiated from cervical NBL and RMSA.
c. Infant desmoid-type fibromatosis is found in muscles, aponeuroses, shoulders, the head and neck, and the upper limbs.
d. Cranial fasciitis can grow rapidly and infiltrate the skull bone.
e. Fibrous hamartoma of infancy (which is congenital in 20% of cases) develops in the cutaneous tissue of the shoulders, arms, thighs, armpits and inguinal areas. It is composed of fibrous, adipose and immature mesenchymal cells, and is treated with surgery.

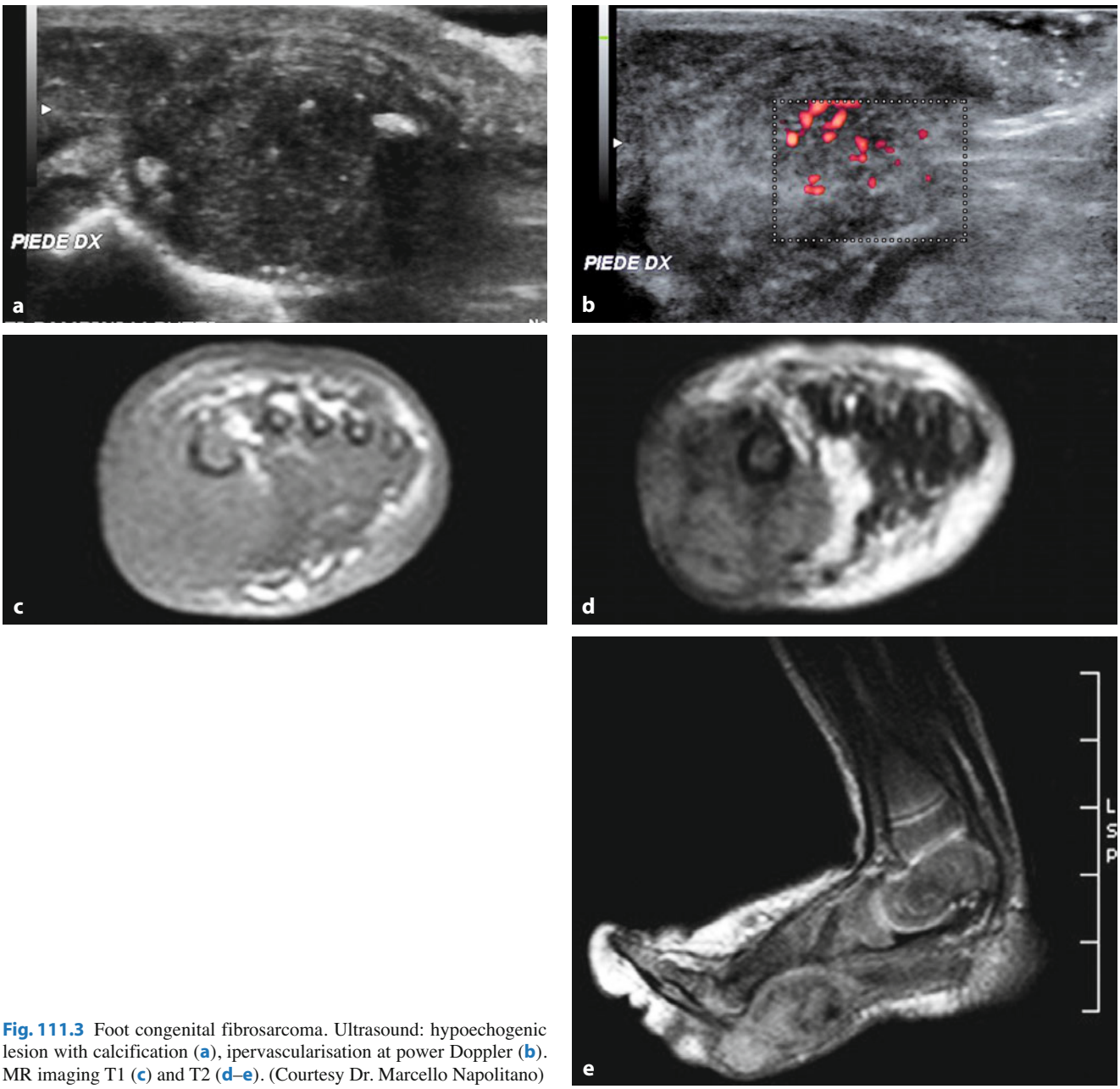

Fig. 111.3 Foot congenital fibrosarcoma. Ultrasound: hypoechogenic lesion with calcification (**a**), ipervascularisation at power Doppler (**b**). MR imaging T1 (**c**) and T2 (**d**–**e**). (Courtesy Dr. Marcello Napolitano)

111.2.4 Kidney Tumors

Neonatal kidney masses are benign in more than 40% of cases. Congenital mesoblastic nephroma, Wilms' tumor and RT are, in order of prevalence, the main perinatal renal neoplasms, but clear cell sarcoma of the kidney can also occur [5, 6, 15–17].

111.2.4.1 Congenital Mesoblastic Nephroma

These account for two thirds of all solid tumors in newborns. It is a mesenchymal tumor resembling infantile fibromatosis. It is generally benign and curable with surgery alone. It can be diagnosed before birth by US, and it has no imaging characteristics to distinguish it from Wilms' tumor, even in the postnatal phase. It is more common in males.

In addition to the abdominal mass, there may be symptoms such as hypertension, hematuria and vomiting. The tumor can grow beyond the renal capsule and invade the surrounding tissues. Local recurrences are observed if the tumor is ruptured during surgery. Distant metastases are rare, especially in the cellular type. The survival rate is better than 90%. Drugs are used for local recurrences or metastases, which however are seldom seen.

111.2.4.2 Wilms' Tumor (WT)

This occurs in nephrogenic residues persisting after the 36th week of gestation. There are no reports in the literature of WT being identified in the newborn carrying the correlated malformative syndrome, but babies with aniridia, hemihypertrophy or Beckwith-Wiedemann syndrome (organomegaly, macroglossia, hypoglycemia at birth) should be monitored to enable the tumor to be diagnosed before the onset of symptoms. The clinical sign of WT consists of an abdominal mass, which must be differentiated from other masses using US/CT/MR (Fig. 111.4). Surgery is the therapeutic procedure of choice. In addition to establishing the stage, histopathology should give information on the presence of anaplastic areas in tumor specimens, which suggest a poor prognosis.

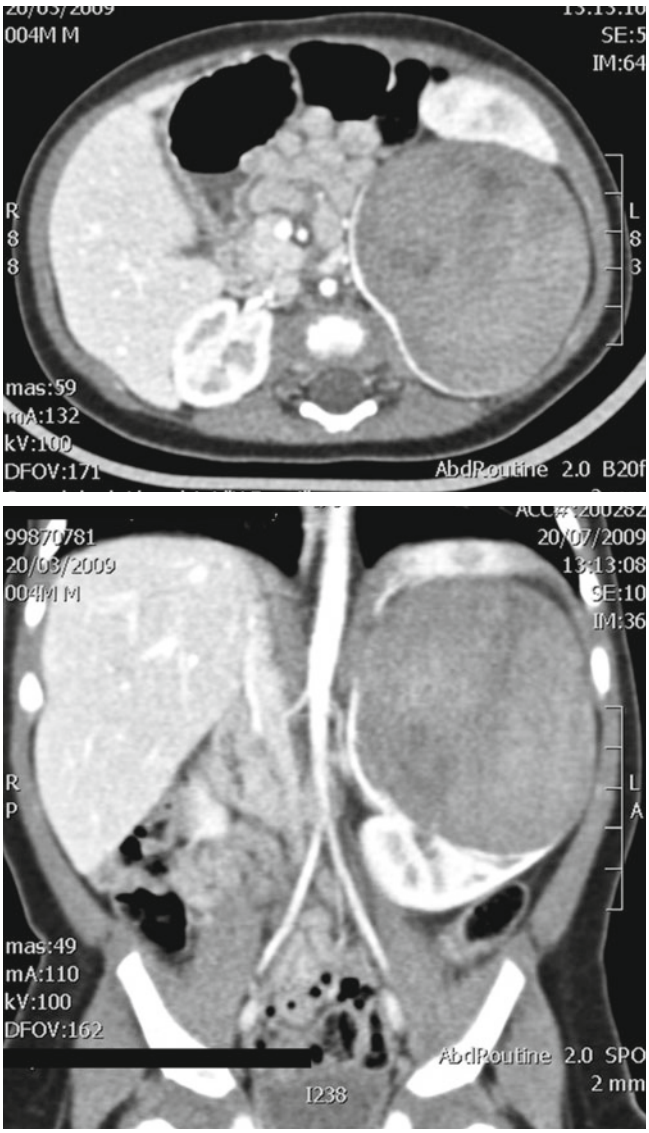

Fig. 111.4 CT imaging of Wilms' tumor with huge solid mass of left kidney

Staging methods have been based on the cooperative protocols of the COG (Children's Oncology Group) and SIOP (International Society of Pediatric Oncology). In the newborn, the tumor is generally detected in stages 1 or 2 and has a favorable histology (no anaplasia). The drugs used postoperatively include vincristine, actinomycin D and/or adriamycin, depending on the stage of disease. Ifosfamide and carboplatin are used in cases with metastases. The prognosis is extremely good (90%).

111.2.4.3 Atypical Teratoid Rhabdoid Tumor and Clear Cell Sarcoma of the Kidney

Both these diseases demand surgery whenever possible but metastases often occur. Chemotherapy has no influence on the prognosis, which is poor.

111.2.5 Brain Tumors

Congenital and neonatal CNS tumors represent 1% of all CNS tumors, and differ in terms of site, histology, clinical behavior and prognosis from those seen in older children. The most common site of onset is supratentorial. Benign cases (which are more common) may suggest malignancy due to their size, since they often cannot be removed. The most common brain tumor is a teratoma, followed by glioma, supratentorial PNET (primitive neuroectodermal tumors) (Fig. 111.5) and medulloblastoma; these last two may coincide with metastatic deposits along the spine.

All malignant CNS tumor types occurring in infants are seen in the newborn too (teratoma 50%, astrocytoma 16%, medulloblastoma 8%, choroid plexus papilloma 7%, ependymoma 3%, unclassified glioma 22%). Symptoms are unusual, macrocephaly (caused by hydrocephalus or the volume of the tumor) being the most significant clinical sign. The fontanel may pulsate. The tumor may displace normal brain without infiltrating it, which explains the absence of focal symptoms. Vomiting and papilledema are late signs, and are due to the increasing size of the neoplasm. Non-specific symptoms, such as lethargy, fever and gastrointestinal disorders, are often misinterpreted, causing delay in the correct diagnosis. MR of the brain and spine provide information on the tumor's size and its benign or malignant nature. Hemorrhagic episodes related to the neoplasm may simulate brain hemorrhage or even cause a massive brain hemorrhage. Treatment options must be discussed on a case by case basis and depend on the site and size of the lesion, imaging findings and the newborn's general conditions. Surgery may be curative in patients with benign teratoma and ependymoma. Cases of PNET and medulloblastoma require pharmacological treatment. Surgery is often only of diagnostic value. Given the poor prognosis (the survival rate is 28% in the

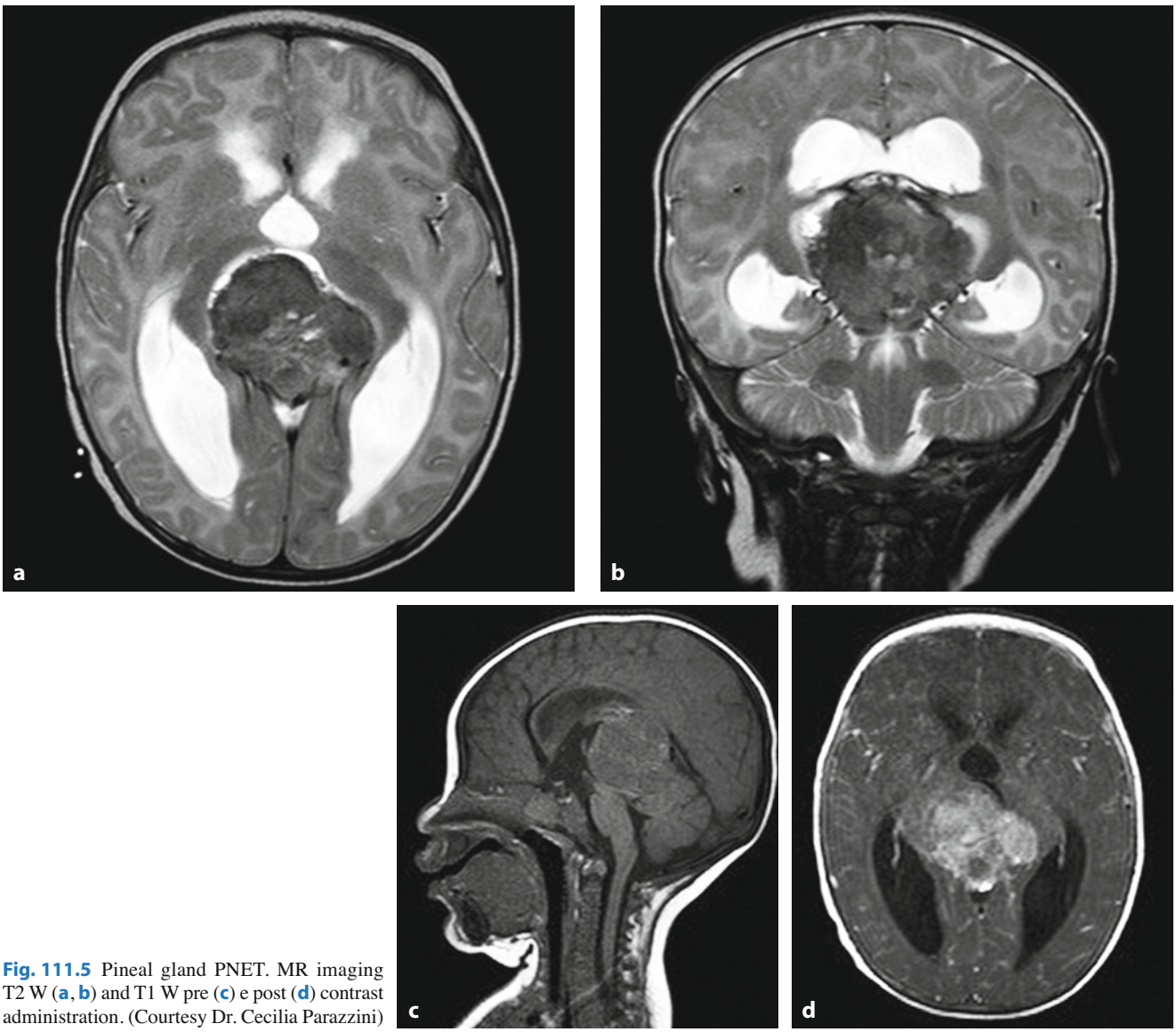

Fig. 111.5 Pineal gland PNET. MR imaging T2 W (**a**, **b**) and T1 W pre (**c**) e post (**d**) contrast administration. (Courtesy Dr. Cecilia Parazzini)

most comprehensive case studies in the literature, which include both benign and malignant lesions), non-intervention may often be the best option. An open and frank discussion between care-givers and relatives is essential before any treatment decisions are made [5, 18, 19].

111.2.6 Liver Tumors

Neonatal liver neoplasms are usually benign (hemangioma 60%, mesenchymal hamartoma 23%, hepatoblastoma 17%). Infant hemangioma is a benign vascular neoplasm, that is generally discovered incidentally during abdominal US for other reasons. There may be no clinical signs of its presence and it may not evolve, but extensive hepatic involvement may be a cause of severe and life-threatening cardiovascular complications [5, 20].

111.2.6.1 Hepatoblastoma (HB)

This is an embryonic neoplasm with variably differentiated epithelial and embryonic tissue with both embryonic and fetal components. It can be found prenatally and may be responsible for polyhydramnios and premature birth. While its association with malformative and genetic syndromes (hemihypertrophy, Beckwith-Wiedemann syndrome, familial polyposis)

is well known, hepatoblastoma has not been described during the prenatal period.

The clinical signs are abdominal distension and hepatomegaly. After imaging tests (MR/CT), the diagnosis is confirmed by a liver biopsy and abnormally high levels of alpha-fetoprotein, which represents a tumor marker. AFP is the major protein produced by fetal liver. AFP levels are markedly elevated in more than 90% of HB. One must relate the AFP values to the physiological AFP levels of premature newborns and infants. The SIOP protocol relies on primary cisplatin chemotherapy, followed by resection. Hepatoblastomas amenable to surgery have a more than 90% favorable prognosis. Liver transplantation should be considered in cases in which hepatic resection is unfeasible.

111.2.7 Leukemias

Leukemia is the most lethal disease of the newborn. It causes fetal hydrops and death, especially in Down syndrome patients. Leukemia in the newborn differs in clinical, hematological, molecular and biological aspects from the disease in older children.

Acute non-lymphoblastic leukemia (ANLL) is more common (65%) than acute lymphoblastic leukemia (ALL) and the former has a better prognosis in cases achieving remission (the survival rate is 24% for ANLL and 10% for ALL). The clinical findings include hepatosplenomegaly and skin lesions; the CNS is sometimes affected. Bone marrow cytomorphological, immunophenotypic and cytogenetic investigations are mandatory for diagnostic purposes. Congenital leukemia is CD10-negative and may be positive for markers in both the lymphoid and the myeloid lines. There is a rearrangement of the MLL (mixed linear leukemia) HRX gene on chromosome 11q23 in 50% of cases of congenital leukemia (both ANLL and ALL), and this is an unfavorable prognostic sign at all ages. Despite its unfavorable prognosis, ANLL may sometimes regress, though it may recur later on. It is important to distinguish congenital leukemia from florid neonatal leukemoid reactions, which are benign conditions defined as "transient abnormal myelopoieses", or "transient myeloproliferative disorders" (TMD), and characterized by hepatosplenomegaly and the presence of myeloid and lymphoid immature cells. The differential diagnosis must also consider fetal erythroblastosis and viral and bacterial infections. TMD differs from congenital leukemia in that the hemoglobin and platelet counts are normal, and blasts in the bone marrow are less than 15%, despite large numbers of white cells and myeloblasts in the peripheral blood. In these benign forms, the newborn's general condition is good and no specific therapy is required. In TMD, hematological indices return to normal over a period of time that may last from a few weeks to 1–2 months. Treatment guidelines for neonatal leukemia are the same as for older children, paying appropriate attention to the dosage of drugs involved, which must be administered in specialized hematological departments. Where feasible, high-dose therapeutic programs followed by hematopoietic cell transplants improve the remission and survival rates, which are currently about 20% [21–24].

111.2.8 Retinoblastoma (RB)

Newborn babies with retinoblastoma lack the normal "red reflex" when undergoing ophthalmoscopic examination, which is a part of routine newborn screening. A family history of retinoblastoma mandates a complete ophthalmic investigation because of the risk of bilateral disease. Familial cases (40%) are caused by a hereditary germinal mutation of the RB1 gene on chromosome 13q14. In such cases, DNA analysis identifies the individuals at risk. Hereditary cases are more common in the newborn, while non-hereditary, unilateral cases are seen more often in older children, who have a "de novo" RB gene mutation. Therapy is based on the outcome of a complete ophthalmic examination and MR can determine the extent of the lesion. The extent of retinal involvement is decribed as a percentage of the total retinal area. For localized forms, laser therapy and cryotherapy, combined with CHT using cisplatin and vepesid, are associated with the best prognosis for vision and survival. Surgery is limited to conditions affecting the extraocular areas, while radiotherapy (which was once used to prevent enucleation) has been abandoned because of the risk of secondary radio-induced tumors in adulthood. Therapy must be coordinated by oncologists and specialized ophthalmologists [2, 25].

111.2.9 Very Rare Congenital Tumors

111.2.9.1 Melanocytic Neuroectodermal Tumor (Melanotic Progonoma)

This tumor of neuroectodermal origin is characteristically located in the jaw, while other locations include the mediastinum and brain. It may be malignant and produce metastases. Surgery and chemotherapy are used, depending on the site of the lesion and the mitotic index.

111.2.9.2 Congenital Melanocytic Nevi

This condition is found in 1% of newborns. Giant forms are extremely rare and may contain nodular areas with a different mesenchymal cellular component. They sometimes resemble melanomas. Giant nevi undergo malignant transformation in

2–5% of cases. Fatal cases of congenital melanoma, with an intracranial involvement, can also occur.

111.2.9.3 Langerhans Cell Histiocytosis (LCH)

LCH comes half-way between malignant and histiocytic disorders. It affects one in a million newborns and 60% are cases of disseminated disease (with multiple organ involvement) while 40% are only cutaneous conditions. Gastrointestinal disease causes diarrhea, vomiting, and protein-losing enteropathy. The diagnosis of LCH requires a positive outcome of the CD1a immunohistochemical test. Depending on the extent of the disease, treatment may include steroids and vinblastine, with or without methotrexate.

The survival rate is 50% in cases of multiple-organ involvement, while it is more than 90% in patients with isolated disease [5, 26].

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112

Fetal Infections: Cytomegalovirus, Herpes Simplex, and Varicella

Stuart P. Adler and Giovanni Nigro

112.1 Introduction

This chapter reviews three herpes viruses that cause infections of the fetus and/or newborn. These are herpes virus V, also called cytomegalovirus (CMV), herpes virus I and II, also called herpes simplex virus 1 and 2 (HSV), and herpes virus III, also called varicella-zoster virus (VZV). With the possible exception of HSV, CMV and VZV may produce severe fetal disease following a primary maternal infection during pregnancy when, in the absence of maternal immunity, these organisms are carried in the bloodstream to the placenta and then on to the fetus. With CMV and VZV, primary maternal infection during pregnancy does not always result in intrauterine infection of the fetus; and when intrauterine infection does occur, severe fetal disease does not always follow. Most commonly, infants infected in utero with CMV appear normal at birth. Chronic persistent infection with each of these herpes viruses causes progressive disease with significant developmental abnormalities which become apparent over the first several years of life. In general, infection of the mother with these viruses and the development of immunity prior to conception protects the fetus either from infection or from the severe disease both in utero and after birth for few months. With CMV, for example, women immune to the virus prior to pregnancy may deliver infants with intrauterine acquired infection but, with rare exceptions, congenital cytomegalic inclusion disease occurs only as the result of a primary infection during pregnancy.

Along with maternal immunity, another important factor that affects both the frequency of transplacental transmission and the severity of disease, is the gestational age of the fetus when the woman becomes infected. Infections with HSV 2 are for most part acquired during delivery from virus in the maternal cervical-vaginal tract. Maternal immunity to HSV 2 prior to delivery protects the newborn from severe or fatal disease due to HSV infection of the newborn. HSV 1 is a virus which is usually orally transmitted but may infect newborns and produce severe disease in the absence of maternal immunity.

Another influence on the severity and the manifestations of an intrauterine infection is the tissue tropism of each virus. Tissue tropism is the affinity of a particular microbe for specific cell types or tissues. Most of the microbes causing intrauterine infection replicate in all organs and tissues, hence causing disease in any or all organs or tissues. An example is VZV which is tropic for neural tissue, and many of the manifestations of severe intrauterine infection with VZV arise from effects upon the developing nervous system.

112.2 Cytomegalovirus

Human cytomegalovirus (CMV) contains within its capsid a double stranded DNA molecule of 150 million molecular weight. This DNA molecule contains at least several hundred genes and is the largest genome of any known virus. CMV viral particles are structurally similar to other human herpes viruses. The virus has a 65 nanometer inner core containing the viral DNA. The inner core is within an icosahedral protein capsid comprised of 162 capsomeres. This is in turn surrounded by a tegument layer and an outer enveloped membrane containing glycoproteins. The envelope glycoproteins are antigenic and are responsible for generating an immune response. The majority of the neutralizing antibodies induced by CMV antigens are directed against the major CMV glycoproteins. Cellular immune responses such as cytotoxic T-cells are directed primarily against the major tegument protein, pp65. There is only a single serotype of CMV. Thus antibodies induced by one viral isolate cross-react with most or all epidemiologically unrelated isolates. Genetically, however, there are probably thousands of different isolates. Each isolate of CMV differs genetically from all other epidemiologically unrelated isolates. CMV infects nearly all humans

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

but infrequently causes disease. CMV causes serious disease in those with impaired immunity associated with immunosuppression due to medical therapy, AIDS, or in the fetus with its immature immune system [1].

112.2.1 Epidemiology

In undeveloped areas or among those in lower socioeconomic groups, between 0.5 and 2% of all newborns are congenitally infected with CMV and by 5 years of age the majority of children have acquired the infection. Within the first year of life, for infants not congenitally infected, CMV is usually acquired perinatally by contact with maternal cervical-vaginal secretions or postnatally from seropositive mothers via breast milk. Approximately 30% of seropositive mothers shed CMV in breast milk and approximately 70% of infants of mothers excreting CMV in the milk will be infected perinatally. The infection is usually asymptomatic in term neonates, while that in preterm infants may be symptomatic. Heating the milk up to 62°C for 5 seconds inactivates the virus while saving its immunologic and nutritional properties.

Between 6 and 12% of seropositive mothers transmit CMV to their newborns via cervical-vaginal secretions. After a primary infection, CMV is shed for prolonged periods. Adults may shed the virus for several weeks or months and intermittently thereafter, but infants and young children shed CMV in saliva and urine for many months or years. Thus, those infants not acquiring CMV from maternal sources presumably acquire the infection from frequent contact with numerous other children shedding CMV. In developing areas with infection acquired early in life, CMV rarely causes acute or chronic disease [2–4].

In developed nations or among those in middle or upper socioeconomic groups, the epidemiology of CMV usually differs from that in undeveloped areas or among those in lower socioeconomic groups. In the United States, for example, although nearly all individuals eventually acquire a CMV infection, infection rates average between 1 and 2% per year. In the US, between 40 and 70% of women between 15 and 45 years of age have antibodies to CMV (seropositive), and seropositivity rates vary by race. Seroprevalence rates are significantly higher among African-Americans than Caucasians [5].

112.2.2 Pathogenesis

Worldwide, approximately 1% of newborns are infected with CMV in utero and excrete virus at birth, but approximately 10% of these infants have congenital CMV disease at birth or develop mental retardation or deafness. Of the 90% of congenitally infected infants who are without symptoms at birth, approximately 85% develop normally. Why do only 10% of infants congenitally infected with CMV have symptoms at birth, and why do approximately 15% of infants without disease at birth develop postnatal sequelae such as mental retardation and deafness? With occasional exceptions, the answer is that infants born of women acquiring a primary infection during pregnancy are those at highest risk for developing congenital disease.

Maternal-fetal CMV transmission as well as neonatal disease expression may be enhanced by transient cellular and humoral immunodepression occurring in the second and third trimesters of pregnancy. While the frequency of fetal infection by primary maternal infection increases with gestational age from about 30 to 60 %, early CMV transmission is correlated with poor outcome. If infection occurs in the 6 months before conception, transmission to the fetus and symptoms at birth will occur at a lower rate. The prevalence of infections following recurrent CMV infection in pregnancy is considered to be lower than 2%, but higher rates have been reported. Pre-existing humoral immunity completely protects seropositive women against reinfection or reactivation at a rate of 75–80%. Neutralizing titers and IgG avidity towards CMV are both inversely correlated with fetal transmission, while the role of cellular immunity is essential in modulating viral transmission and pathogenicity in pregnancy. A major pathogenic site of CMV is the placenta, which may be impaired in its capacity to provide oxygen and nutrients to the developing fetus [6–13].

Of congenitally infected newborns whose mothers acquired a primary CMV infection during pregnancy, approximately one-third will either have disease at birth or develop significant sequelae (Fig. 112.1). The risk for symptomatic infection appears to be greatest when primary maternal infection occurs in the first half of pregnancy, but congenital disease may also occur among infants born of mothers with a primary infection in the second half of pregnancy. If infection occurs in the 6 months before conception, transmission to the fetus and symptoms at birth will occur at a lower rate. Because primary maternal infection is responsible for the majority of congenital CMV disease, symptomatic infection is most frequent in areas with a high proportion of seronegative women frequently exposed to the virus. Primary maternal CMV infections during pregnancy are seldom recognized and many infants who develop neurologic sequelae following a primary maternal infection in early pregnancy are asymptomatic at birth. Thus, the precise number of affected infants born annually in any country is unknown. Estimates for the US are that between 1 and 4% of 4 million pregnant women acquire a primary CMV infection in the US annually and that up to 8000 newborns annually may develop significant handicaps caused by congenital CMV infection [3–5].

112.2.3 Clinical Manifestations

Cytomegalovirus infects the placenta and causes placental dysfunction manifested by an enlarged placenta and placental inflammation. Manifestions of placental dysfunction in the

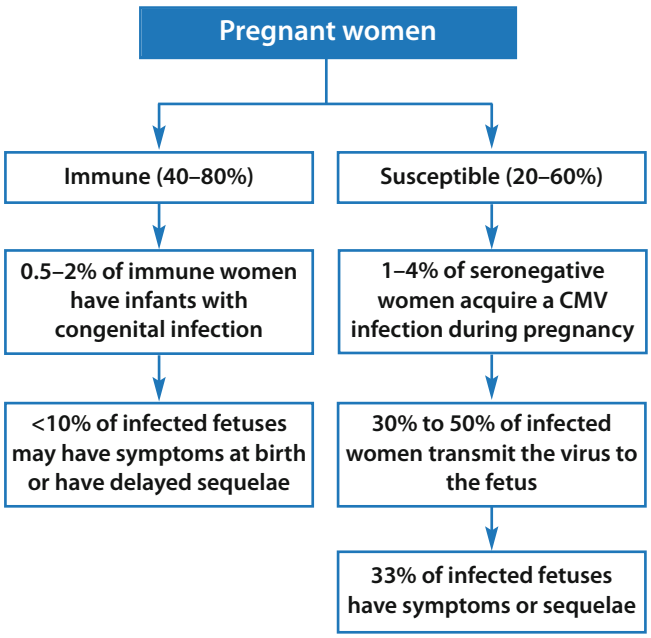

Fig. 112.1 Relationship of maternal immunity to disease caused by congenital CMV infection

newborn include growth restriction and problems with the reticuloendothelial and the central nervous system. Reticuloendothelial problems include hepatosplenomegaly and hepatitis, hemolytic anemia, thrombocytopenia, and direct hyperbilirubinemia. Central nervous system (CNS) involvement is often manifested by microcephaly and intracranial calcifications, typically periventricular. Alterations of neuronal migration, which occur between the third and fourth month of pregnancy, cause cerebral dysplasias (polymicrogyria, pachygyria, lissencencephaly, schizencephaly).

CMV is the only congenitally transmitted pathogen capable of causing alterated gyral development, the pathogenesis of which include both teratogenic and encephaloclastic mechanisms. Table 112.1 lists the more common features characteristic of infants who are symptomatic at birth. At birth chorioretinitis or intracranial calcifications or other abnormalities on a computerized axial tomography (CAT) scan or ultrasound of the head are associated with a poor neurologic prognosis, but none of the other manifestations of congenital infection accurately predict the severity of postnatal mental retardation or hearing deficit [5, 8, 13].

Approximately 15% of infants asymptomatic at birth will develop significant hearing deficits or mental retardation within the first 5 years of life. This may occur because CMV causes a chronic persistent infection. Symptomatic children are often viremic at birth and viremia may persist for the first few months of life. Both symptomatic and asymptomatic children will excrete CMV in urine and/or saliva for up to five years postnatally. At birth symptomatic children shed higher titers of virus (up to 10^6 PFU/mL of urine) than asymptomatic infants. Hearing deficit, either mild or absent at birth, may develop or progressively worsen over the first several years of life, perhaps as a result of the chronic CMV infection. Therefore, parents of congenitally infected children, including those without symptoms, should not be given a prognosis at birth. When hearing deficit is profound, however, it is not generally reversible. The severity of mental retardation and developmental delay, which are the most serious neurological sequelae of this infection, are difficult to predict at birth. Most children who are symptomatic at birth will have some degree of mental retardation or developmental delay; however, IQ scores may approach 100 or higher in these children. Thus, the level of impairment in these children may be relatively mild or inapparent. Congenital CMV infection should be suspected in any infant with one or more of the manifestations listed in Table 112.1 [3, 5, 12].

Transmission of CMV within the hospital occurs very infrequently. In numerous studies, CMV transmission from infected patients to hospital personnel has not been observed. Patient-to-patient transmission is rare and may occur when patients are hospitalized in close proximity for prolonged periods. Thus, CMV seronegative women caring for hospitalized young children or for other patients who may be excreting CMV can be reassured that there is minimal risk of acquiring CMV from patients. CMV may be transmitted by transfusion of whole blood and is located in the white cell fraction. For immunocompromised patients requiring transfusion the risk of CMV acquisition from a blood donor is eliminated either by selecting donors who are CMV seronegative or using blood products with white cells removed [3, 5].

Table 112.1 Common features in children with symptomatic congenital CMV infections

1. Unexplained hepatomegaly associated with abnormal liver function tests
2. Unexplained splenomegaly
3. Unexplained hyperbilirubinemia (usually direct > indirect)
4. Ocular findings, including chorioretinitis, strabismus, optic atrophy, microphthalmia, cataracts, or retinal necrosis
5. Microcephaly
6. Cerebral calcifications
7. Hemolytic anemia

112.2.4 Diagnosis

Without prospective serologic testing of pregnant women the diagnosis of a primary maternal infection during pregnancy is often difficult and is often only undertaken if signs and symptoms of either maternal infection or fetal disease become apparent. Symptoms of maternal CMV infection occur in about 25% of pregnant women infected during pregnancy. Maternal symptoms include a flu-like syndrome, myalgia, and asthenia with or without fever. There may also be a lymphocytosis

and/or elevated liver enzymes in about 50% of patients. Signs of fetal infection on maternal ultrasound include growth restriction, microcephaly, echodensities in solid organs or the intestines, or an enlarged placenta [13–17].

The best way to diagnosis of a maternal infection is by maternal seroconversion, however, this rarely occurs unless universal screening of pregnant women is standard practice in a particular country. Hence initial seronegative sera are rarely available. The detection of IgM antibodies in maternal sera may be helpful but although IgM antibodies to CMV occur in all primary infections they may also occur after reactivations or reinfections. After a primary infection IgM remains for many months. Thus IgM to CMV in a single serum of a pregnant woman does not alone establish a primary CMV infection during pregnancy. Antibody avidity, a measure of tightness of antibody binding to its target antigen, increases in the first weeks after a primary infection. Low avidity IgG antibodies to CMV persist for up to 20 weeks after a primary CMV infection. These low avidity antibodies are then replaced by high avidity antibodies (> 60% binding in presence of 5M urea). Thus the combination of IgM antibodies to CMV and low avidity IgG antibodies to CMV plus maternal or fetal symptoms argues for the diagnoses of a primary maternal infection [18–19].

Amniotic fluid may be a helpful adjunct in diagnosis. While viral culture of the amniotic fluid may yield false negative results, polymerase chain reaction (PCR), especially after 21 weeks gestation, is both sensitive and specific for fetal infection. A diagnosis of fetal CMV infection alone is insufficient to predict newborn disease, however, fetal abnormalities or placental enlargement by ultrasound predict newborn disease and long-term sequelae [7–13].

In the newborn the diagnosis for congenital CMV is established with a positive urine culture within the first 3 weeks of life. With the shell vial assay, which detects the immediate early protein of CMV expressed on cultured fibroblasts within 48 hours of inoculation, a diagnosis of congenital CMV infection can usually be obtained with 48–72 hours of life. Because congenital infection with CMV occurs in up to 2% of all newborns and because 85–90% of these babies will develop normally, a positive urine alone does not establish a diagnosis of symptomatic congenital CMV infection. One must also exclude congenital toxoplasmosis and syphilis [5].

112.2.5 Newborn Evaluation

If a congenital CMV infection is established or suspected, an evaluation should include measurement of transaminases and direct bilirubin. If liver function is abnormal, it should be monitored until near normal, which may take several months. A complete blood count should be obtained as some infants have a hemolytic anemia and almost all will have low platelets which may be mild or severe. Thrombocytopenia which is due to reduced platelet production in utero associated with CMV-induced megakaryocytic lysis and intrauterine hypoxia usually resolves in 1–2 weeks.

A lumbar puncture and head CT scan or head ultrasound should be obtained. The presence of cellular pleocytosis and elevated protein in the cerebral spinal fluid (CSF) or periventricular calcifications indicates CNS involvement. Other manifestations include ventriculomegaly and/or hydrocephalus. Cerebral dysplasias or atrophy may be seen by magnetic resonance imaging (MRI). An ophthalmologic exam should be obtained to evaluate for chorioretinitis.

Hearing deficit is an important consequence of congenital CMV infection and is the main cause of non-genetic sensorineural hearing loss accounting for about 23% of all hearing deficit that occurs before language development. Given this and the fact that hearing loss may be fluctuating and progressive, a complete audiologic exam should be performed soon after diagnosis with frequent subsequent exams over the first few years of life. Most infected infants will develop hearing loss in the first 2 years of life, although hearing deficit may develop as late as 4 years of age. Audioevoked potentials or brainstem evoked potential testing are the most accurate tests [20, 21].

112.2.6 Treatment

112.2.6.1 Prenatal Therapy

An effective therapy for fetal CMV disease is not yet available, in spite of relevant advances in the diagnostic procedures of maternal-fetal CMV infection. Pregnancy termination is offered as a management option when injured, or even only infected, fetuses are identified by ultrasonography or amniocentesis, respectively. A few case reports and one study have focused attention on the safe administration of oral ganciclovir or valacyclovir, respectively, to mothers of CMV-infected fetuses. The favorable use of CMV immunoglobulin intravenously to the mother and intramniotically to treat or prevent fetal CMV infection has been reported in a few case reports and in one study, which evidenced favorable clinical and immunological results. The placenta is probably the key site of activity of immunoglobulin, as shown by studies in guinea pigs and humans, in which placental enlargement due to CMV infection can be reduced by immunoglobulins [22–25].

112.2.6.2 Postnatal Therapy

CMV immunoglobulin was shown to be protective against CMV disease by studies on neonatal transfusions. Antiviral treatment of neonates or infants with congenital CMV infection is generally based on the use of ganciclovir, which has been given at different endpoints and regimens (both dosage and duration of therapy), and for different clinical manifestations. This drug is active only after phosphorylation to

ganciclovir-triphosphate, which substitutes guanosine-triphosphate in the viral DNA polymerase, with consequent inhibition of CMV replication. Since the first phosphorylation requires the presence of the CMV-encoded (UL 97 gene) phosphotransferase, ganciclovir can display its activity mostly in the CMV-infected cells. Besides being a possible consequence of CMV infection, neutropenia and increased aminotransferase levels may be associated with ganciclovir therapy, particularly in immunodepressed patients. A longer duration of antiviral therapy has been associated with favorable outcome more frequently than shorter courses, probably related to the longer inhibition of CMV. In fact, this virus reactivates soon after stopping therapy, and some of the favorable results obtained during ganciclovir therapy may be lost. Protracted and repeated courses of oral ganciclovir were useful for therapy of several CMV-related diseases, including neurologic and gastro-intestinal manifestations [21, 24–26].

112.2.7 Prevention

As CMV is a major cause of childhood deafness and neurological handicap, routine antepartum screening should be very useful, but appears to be impracticable. To avoid difficulties in differentiating recurrent from primary infections and problematic counseling when positive IgM antibodies are detected, serial examinations of CMV-specific IgG and IgM antibodies at least in the first two trimesters, particularly in women at high risk, such as daycare workers, may be suggested. In such a way, it is possible to reveal as soon as possible both primary (by seroconversion and high IgM levels) and recurrent (by significant IgG increase concomitantly or not with positive IgM antibodies) infections. To prevent possible viral transmission to the fetus, women with primary infection could receive hyperimmunoglobulin. Appropriate hygienic measures can prevent CMV transmission. The most aggressive preventive approach requires counseling of parents with seropositive children in daycare centers: seronegative parents should be instructed to frequently handwash after handling diapers and material contaminated with secretions and cautioned about the potential risk of intimate contact, especially mouth-to-mouth [20]. Seronegative women with multiple sexual partners and a history of sexually transmitted diseases should be counseled about the use of condoms. Since the detection of CMV DNA in CSF has been associated with developmental delay, this evaluation could be used in the future to identify infants who are at risk for neurodevelopmental problems and who may therefore benefit from antiviral therapy. A vaccine containing recombinant cytomegalovirus (CMV) glycoprotein B subunit antigen combined with MF59 adjuvant showed the potential to decrease of 50% incident cases of maternal and congenital CMV infection. Other vaccines, including live attenuated vaccines, other subunit vaccines, or vaccine candidates formulated with adjuvants other than MF59 are under evaluation [24, 27, 28].

112.3 Infection with Herpes Simplex Virus, Serotype 1 and 2

112.3.1 Epidemiology

HSV is another member of the Herpesviridae family, including two closely related virus types (1 and 2), which are transmitted through mucosal cells or cutaneous lesions and migrate to the nerve tissues, in which they persist in a latent state. HSV-1 predominates in the orofacial manifestations and is typically found in the trigeminal ganglia, whereas HSV-2 is most commonly detected in the lumbosacral ganglia. Nevertheless, these viruses can infect both orofacial areas and the genital tract, where they can reactivate either asymptomatically or with vesicular lesions [29, 30].

Vertical transmission is more likely to occur after primary maternal infection, but recurrent genital infections are more frequent and, consequently, they are the most common cause of neonatal herpes infections. The great majority of recurrent genital herpes are due to HSV-2 because this virus reactivates in the genital area more frequently than HSV-1 (7 versus 2%, respectively). In the asymptomatic period between clinical outbreaks of genital herpes, HSV can reactivate periodically in latently infected cells of sensory ganglia travelling via the neuronal axons back to the genital mucosa, without clinical signs or symptoms (“asymptomatic virus shedding”). The majority of sexual HSV transmission occurs during asymptomatic shedding because the patients are unaware of the viral presence [30–32].

Newborns may acquire infections with HSV in utero, peripartum at delivery, or postpartum. Approximately 5% of all newborn infections with HSV are acquired in utero, 85% are acquired at birth by contact with infected maternal lesions or asymptomatic cervical shedding of HSV, and 10% are acquired postpartum from environmental sources, such as scalp electrodes or individuals with oral HSV infections. Intrauterine infections with HSV occur with a frequency of from 1 in 200,000 to 1 in 300,000 live births as opposed to peripartum infections estimated at 1:5000. In utero infection during the first 20 weeks of gestation is associated with the highest infant morbidity and mortality, including stillbirths and spontaneous abortions [29, 31].

112.3.2 Intrauterine Infections: Pathogenesis

Although the exact route by which infants acquire intrauterine HSV infections is unknown, there is evidence that most infants are infected by virus ascending through the chorioamnionic membrane rather than from a maternal viremia and transplacental passage of HSV to the fetus. The first observation is that during both recurrent and primary maternal HSV infections, maternal viremia does not occur. The second is that HSV is commonly present in the cervical-vaginal tract,

and this is the usual site of primary or secondary HSV infection in women who deliver infants with intrauterine HSV infection. Although women with a primary HSV infection deliver infants with the most severe form of intrauterine infection, these primary infections are still in the cervical-vaginal tract and viremia does not occur. The third observation is that even when a woman with viremia develops disseminated HSV disease during pregnancy, the majority of fetuses born of these women die from maternal complications, but not as a consequence of intrauterine HSV. Surviving fetuses of women with widely disseminated maternal disease usually have no evidence of HSV disease at delivery. This suggests that the occurrence of maternal viremia is not necessary for intrauterine infection with HSV [29, 31].

The fourth fact is that most infants born with intrauterine HSV infection have cutaneous disease with scarring. The chronicity of the skin infection suggests that, as in postpartum and peripartum acquired infections, the skin or the mucous membranes are the first sites of HSV infection, followed by fetal viremia and subseqent central nervous system infection. Another fact suggesting an ascending route of infection is that when HSV is in the placenta, there is extensive fetal involvement, suggesting passage of virus was from fetus to placenta. In infants who survive placental infection there is usually evidence of disseminated organ dysfunction. The final fact suggesting an ascending route of infection is that nearly all infants with intrauterine infection are infected with HSV-2, the serotype commonly found in the maternal cervical-vaginal tract. If the route of fetal infection were via the maternal blood stream one would anticipate frequent fetal infection with HSV-1, since this serotype is common in pregnant women [31–33].

112.3.3 Intrauterine Infections: Clinical Manifestations

Diagnosis of intrauterine HSV infection requires clinical evidence of HSV infection within the first 48 hours of life and confirmation by recovery of the virus from the newborn. Symptoms are usually present at birth (Table 112.2). The classic symptom triad is skin scarring, hydranencephaly, and chorioretinitis. Skin vesicles and bullae are common. There may also be erythematous maculas that later become vesicles or areas of aplastic or denuded skins, or scars. These may occur at sites of trauma, for example fetal scalp electrode sites. Skin lesions often recur, but do not usually progress to other sites. Dissemination with multi-organ system involvement, if present, includes hepatic (i.e., liver failure), renal (i.e., anuria), and hematologic disorders (i.e., disseminated intravascular coagulopathy). Intrauterine growth restriction and subsequent postnatal growth retardation are also common [34].

When hydranencephaly is absent, microcephaly, intracranial calcifications, hydrocephalus, porencephalus, and subdural or epidural cysts may occur with subsequent blindness, deafness, and mental retardation. Involvement of the eye is common, particularly chorioretinitis with a strabismus. This occurs more frequently with intrauterine HSV infection than with HSV infection acquired peripartum or postpartum. Disseminated infection in infants with intrauterine disease is much less frequent than in infants with perinatally acquired infection because those infants who do develop disseminated disease usually die in utero [34, 35].

Table 112.2 Clinical manifestations of congenital or perinatal infection by herpes simplex virus (HSV)

Congenital HSV infection
Cutaneous lesions: scarring, vesicles, bullae, macular erythema, areas of aplastic or denuded skin
Cerebral: hydranencephaly, microcephaly
Ocular: chorioretinitis, microphtalmia
Perinatal HSV infection
Encephalitis
Pneumonia
Esophagitis
Muco-cutaneous manifestations
Systemic disease

112.3.4 Peri and Postnatal HSV Infections

112.3.4.1 Epidemiology

HSV transmission follows a direct contact with oral or genital secretions, even if asymptomatic. HSV-1 infection occurs early in the low economic level populations, while HSV-2 prevalence rapidly increases with sexual activity. Oral recurrences occur in 20 to 40% of the adult population. The prevalence of neonatal HSV infection is 1/2500–20,000 infants, being caused by HSV-2 in two thirds of the cases [29].

112.3.4.2 Pathogenesis

After entry at mucosal surfaces or abraded skin, HSV proteins bind to several cell receptors of epidermal and dermal cells, in which the virus replicates. In both clinical and subclinical infections, HSV reaches sensory or autonomic nerve endings and is transported intraaxonally to the nerve cell bodies in ganglia, in which initial viral replication takes place. Then, HSV spreads to other mucosal and skin cells by centrifugal migration along peripheral sensory nerves. After primary infection, viral DNA may be found in 10 to 50% of ganglion cells of the initially involved anatomic region. Ultraviolet light, immunodepression and trauma are associated with HSV reactivation but the pathogenic mechanism is unknown [31–33].

112.3.5 Clinical Manifestations

112.3.5.1 Neonatal Herpes

Neonatal herpes includes three major clinical features: oculo-mucocutaneous (50%), encephalic (33%) or systemic manifestations (17%) [9, 30]. Mucocutaneous and ocular signs can occur several days after delivery, and are generally slight: vesicles, which are often grouped, may be purulent and be considered as bacterial bullae. Encephalitis is shown, 2–3 weeks after birth, by fever and irritability or lethargy in the first 2 weeks, then by alterated muscular tone and partial or diffuse convulsions. The cerebro-spinal fluid shows 50–100 leukocytes, decreased glucose but increased protein content. Electroencephalography (EEG) reveals signs of disseminated cerebropathy, CT and MRI scans show focal alterations. The systemic infection is shown by fever, lethargy, hepatic dysfunction, pneumonia, disseminated intravascular coagulopathy and shock. Other signs include seizures, jaundice, and a vesicular exanthema which may be pathognomic. Encephalitis is a common manifestation (60–75%) of the disseminated HSV infection [36, 37].

The rate and severity of neonatal HSV infection depend on the type of maternal genital infection: the frequency is higher after primary than recurrent maternal infection (50 versus < 3%). However, 70% of neonatal HSV infections result from exposure to asymptomatic maternal HSV shedding during delivery. Prolonged rupture of membranes favors neonatal infection: a cesarean section should be chosen. Invasive obstetrical procedure and fetal scalp monitors can cause lesions which are sites of viral inoculation [31, 33, 34].

112.3.5.2 Gingivostomatitis

Gingivostomatitis is predominantly caused by HSV-1 and typically shown by vesiculo-ulcerative lesions on the tongue, lips, gingival, buccal mucosa and the hard and soft palate. Fever, painful oropharyngeal mucositis, cervical lymph-adenitis and fetid breath persist for several days and can cause dehydration because of the difficult feeding even with liquids [36].

112.3.5.3 Keratoconjunctivitis

Keratoconjunctivitis is commonly caused by HSV-1, presents as monolateral follicular conjunctivitis, localized lymph-adenomegaly, palpebral vesicles, photofoby, edema and corneal opacity [37].

112.3.5.4 Encephalitis

Encephalitis is one of the most common acute viral infections of the brain (1/250–500,000 people every year), caused by HSV-1 in > 90% of the cases, predominantly after recurrent infections. If untreated, it is lethal in 60–80% and followed by severe sequelae in > 90% of the surviving patients [32, 37].

112.3.5.5 Cutaneous Herpes

Primary cutaneous herpes infections can be localized in any part of the body, sometimes as an extensive zoster-like manifestation. Recurrences are generally localized on the lips, both mucosal and cutaneous parts. Because the immune system is still developing, then partially immature in early infancy, at this age herpetic recurrences may be more frequent and extensive than in adults. Occasionally, the children may present recurrent stomatitis or aphtosis with or without an erythema [35].

112.3.5.6 Herpes in Immunodepressed Children

Herpes in immunodepressed children occurs as an extensive mucocutaneous or multi-system infection, including Kaposi's varicella-like eruption or *eczema herpeticum*, pneumonia, meningoencephalitis. Children with AIDS may have a rapidly worsening and overwhelming herpetic infection shown by esophagitis, enterocolitis, pneumonia, hepatitis and neurological manifestations [35, 37].

112.3.6 Diagnosis

The HSV infection may be identified directly by detection of the virus or one of its components or indirectly by assaying for specific serum antibodies of the viruses. Direct site-specific methods, such as DNA detection by RT-PCR or viral isolation, are most relevant in patients with active, vesicular lesions at or near a genital site. When lesions have scabbed or are not evident, HSV-1 or HSV-2 infection can be diagnosed indirectly by detection of type-specific IgG against the glycoprotein G of HSV-1 (gG-1) or the glycoprotein G of HSV-2 (gG-2). Indirect (serological) testing can provide useful information in symptomatic patients when direct methods have yielded negative results. The detection of HSV-specific IgM antibodies may be negative or non-specific [32, 33].

Intrauterine HSV infections are diagnosed by virus detection from the infant's samples within the first 48 hours of life. Cultures should not be obtained for HSV early from asymptomatic infants or infants who have none of the signs or symptoms of intrauterine HSV infection because some infants may only be colonized with HSV at birth. Cultures should be obtained from skin lesions, perianally, mucous membranes, eyes, nose, oral pharynx, and ears. More sensitive than culture is the viral DNA detection by PCR from the liquid of vesicles and any other sample, particularly the CSF. A Tzank preparation

or Wright's stain of epithelial cells scrapped from cutaneous lesions may reveal multinucleated giant cells which are associated either with HSV or VZV lesions. After the first 48 hours of life, the isolation of HSV from an asymptomatic newborn means an active infection rather than colonization and requires antiviral therapy. Tissue HSV involvement may be also diagnosed using immuno-hystochemical methods or in situ hybridation. Cerebral imaging and auditory/ophthalmological examination should be performed [3, 32, 33, 37].

112.3.7 Treatment

Specific antiviral therapy with acyclovir is appropriate for infants with either intrauterine or postnatal HSV infections. To treat encephalitis or systemic HSV infection, intravenous acyclovir (10–15 mg/kg three times daily) should be started soon after collection of the samples for virologic confirmation. If acyclovir-resistance develops, ganciclovir or, preferably, foscarnet should be given.

For gingivo-stomatitis or muco-cutaneous lesions, oral acyclovir should be given at the dosage of 200 mg five times daily. The starting time of treatment is crucial for prognosis, especially in case of disseminated infections. HSV infections localized to skin, eyes and mucous membranes are treated for 14 days, whereas CNS or disseminated infections required 21 days of therapy. For recurrent skin lesions, which are frequent after neonatal HSV infection, acyclovir treatment is indicated for weeks to months, at doses of 300 mg/m^2 2–3 times daily [35, 38].

112.3.8 Prognosis

For both intrauterine and postnatal HSV encephalitis and systemic infections, if untreated, the mortality is about 70%. Treatment with acyclovir reduces mortality until to about 30% and to only 5% for neonates with encephalitis. The effect of antiviral therapy on morbidity is unknown; and some treated infants, particularly those with manifestations of central nervous involvement, will be blind, deaf, or profoundly retarded. The outcome is correlated with the virus type and disease classification. Moreover, after a neonatal herpes infection, cutaneous recurrences may occur [39].

112.3.9 Prevention

Numerous approaches, including subunit or peptide vaccines, live virus vectors and DNA vaccines, have been used in developing both prophylactic and therapeutic vaccines, since several antiviral therapies are available to control disease and spread, but these are not completely effective and do not affect latent virus. Currently, neither active nor passive immunization is available for prevention of HSV infections in pregnant women and neonates. However, the developing vaccine strategy should take in account the most important features of herpesvirus family: viral latency, immune escape and high seroprevalence [33, 40].

All pregnant women with genital herpes should be treated with acyclovir or valacyclovir, which were shown not to be associated with teratogenic effects. However, since these drugs are not officially approved for treating pregnant women, patients should give consent about the limited information before the drug is used. The high rate of undiagnosed or asymptomatic HSV infections complicate prevention. Identification of the serostatus of pregnant women during early pregnancy is the first and most important step, to establish their susceptibility to the infection. A history of HSV infection in all pregnant women and their partners should be obtained at the first prenatal visit. Women with a negative personal history of HSV and especially those with a positive history in the male partner, should be strongly advised to have no oral and sexual intercourse at the time of recurrence in order to avoid infection, particularly in the third trimester of pregnancy. The use of condoms throughout pregnancy should be recommended to minimize the risk of viral acquisition, although they are not considered to be a complete barrier for the genital region.

Prophylactic administration of acyclovir or valacyclovir in the third trimester of pregnancy should be provided to all pregnant women with frequent genital herpes outbreaks and with active genital HSV infection near term or at the time of delivery. A careful examination of the vulva, vagina and cervix should be performed on any woman who presents signs or symptoms of HSV infection at the onset of labour. Artificial rupture of membranes should be avoided. All pregnant women who have suspected active genital HSV infection should undergo cesarean section, although membranes are intact. Neonates, born to women with active genital lesions, with a confirmed or suspected HSV infection should be isolated, managed with precautions to avoid direct contact with skin and mucosal lesions, excretions, body fluids and immediately treated with intravenous acyclovir. Since neonatal herpes can also be acquired postnatally, postpartum women, family members and nursery personnel with active herpetic lesions of the mouth, skin or breast should take necessary precautionary measures to prevent direct contact with the neonate and/or should be excluded from the neonatal unit until the lesions are fully healed [29, 31, 34, 35].

112.4 Varicella

112.4.1 Epidemiology

Varicella zoster virus (VZV) is a herpesvirus, which causes chicken pox and shingles. About 5% of women have not had

chicken pox before pregnancy. Women who have had chicken pox before pregnancy are immune and their fetuses are not at risk. The average incidence of varicella in pregnancy ranges 0.7–3%. If a woman becomes infected with VZV during pregnancy, infection of the fetus with the development of a varicella syndrome occurs up to the 26th week of gestation. However, the risk for intrauterine disease after a primary maternal VZV chicken pox is low, about 4%, mostly in the first two trimesters. This is much lower than when a woman acquires primary varicella within a few days of delivery, when the risk for the newborn of acquiring postnatal varicella approaches 20%. The rate of spontaneous abortion by varicella does not exceed that of women without varicella. Pregnant women who contract varicella are at risk of severe pneumonia associated with life-threatening ventilatory compromise and death, apparently more often in the third trimester. If a woman develops zoster, also called shingles, during pregnancy there is no risk to the fetus [3, 41, 42].

112.4.2 Pathogenesis

The VZV virus causes both primary chicken pox and recurrence within an initially infected dermatome. Recurrence which occurs months to decades after a primary infection is called shingles or zoster. VZV infection in a normal person consists of two episodes of viremia. The first episode of viremia follows the replication of the virus at a regional lymph node in the head or neck. This primary viremia results in the seeding and replication of VZV at multiple body sites. After the second stage of viral replication, secondary viremia leads to infection of epithelial cells in the entire body and produces the skin lesions of chicken pox.

Transmission to the fetus occurs with either the primary or secondary viremia. Infection after a primary maternal viremia can cause fetal disease within a day or two of the appearance of skin lesions in the mother. If, however, fetal infection occurs after the secondary maternal viremia, fetal skin lesions do not appear until 10–14 days following the appearance of lesions in the mother.

In the first 20 weeks of gestation, since the fetal organs are embryonic, VZV may cause teratogenic abnormalities. Maternal chicken pox late in gestation may lead high fetal mortality but not structural abnormalities. Spontaneous abortion, fetal demise, and premature delivery can occur at an indetermined but low frequency [43, 44].

112.4.3 Clinical Manifestations

112.4.3.1 Congenital Varicella Syndrome (CVS)

Since VZV has a tropism for peripheral and central neural tissue, the major manifestations of intrauterine varicella infections are those related to the nervous system. The characteristic clinical signs and symptoms (Table 112.3), which also include skin lesions in dermatomal distribution, eye diseases and limb hypoplasia are: zigzag skin lesions and hypopigmented areas, damage to the eye including microphthalmia, cataracts, chorioretinitis and optic atrophy, damage to the cervical-lumbar spinal cord with hypoplasia of the upper and lower extremities with decreased motor and sensory deficits, absent dependent reflexes, anal dysfunction, and Horner's syndrome. Based on the segmental distribution of some clinical features, some lesions might be not consequent to immediate intrauterine varicella but zoster-like VZV reactivations. Cortical damage includes encephalitis with microcephaly, hydrocephaly, intracranial calcifications, and aplasia of the brain. In spite of the initial poor prognosis, a favorable outcome can occur in children with CVS. In addition to the developing disease, prenatally infected infants may develop VZV infection without symptoms: intrauterine infection is more common than fetal disease. The developmental effects of asymptomatic intrauterine VZV infection are unknown [43, 44].

Table 112.3 Congenital Varicella syndrome: principal clinical manifestations

Skin lesions
Microphtalmia, chorioretinitis, cataract, atrophy of the optical nerve
Neurosensorial deafness or hypoacusia
Microcephaly, calcifications, cerebral atrophy, hydrocephalus, mental retardation
Limb hypoplasia or paresis

112.4.3.2 Neonatal Varicella

Maternal varicella between 4–5 days before and 2 days after delivery may be followed by a generalized and severe neonatal varicella, since protective antibodies are not transferred to the infant. The attack rate for the infant ranges 20%. A fatal outcome is more likely if neonatal disease occurs between 5 and 10 days after delivery. Chickenpox occurring after the first 10 days of life is most likely acquired postnatally, and is associated with a low morbidity, since most neonates are protected by maternal antibodies. However, premature infants younger than 28 weeks' gestation or weighing below 1000 g are at increased risk for severe varicella during the first 6 weeks of life [43, 44].

112.4.3.3 Complications of Postnatal Varicella

Otherwise healthy children may have secondary bacterial infections, usually caused by *S. aureus* and *S. pyogenes*, meningoencephalitis, which is prevalent under 5 years or over 20 years of age, acute cerebellar ataxia, Reye syndrome, which is significantly reduced by avoidance of salicylates, transverse

myelitis, hepatitis, thrombocytopenia, arthritis. These clinical manifestations may have a vasculitic or immune-mediated pathogenesis. Immunocompromised children, pregnant women and adults are at high risk of developing a severe and progressive varicella. Varicella pneumonia is the major cause of maternal morbidity and mortality. The risk of progressive disease, including prolonged cutaneous lesions, pneumonia, hepatitis, encephalitis, and disseminated intravascular coagulopathy is up to 50% for children with lymphoproliferative malignancies or solid tumors, particularly if chemotherapy is given during the incubation of varicella. In children with cancer, disseminated VZV infection may also be associated with esophagitis, enterocolitis, pancreatitis, and necrotizing splenitis. Thrombocytopenia and hepatitis are the major clinical complications in kidney transplanted patients. Children receiving high doses of corticosteroids, especially during the incubation period, may develop a severe, even fatal varicella [42–44].

112.4.3.4 Diagnosis

Maternal varicella may be confirmed by detection of specific IgM and IgG antibodies. Fetal ultrasound and MRI at 16–22 weeks' gestation or 5 weeks after infection can show possible signs of CVS. Fetal infection should be confirmed by detection of VZV DNA in the amniotic fluid. Termination of pregnancy may be suggested if serious fetal abnormalities are shown. The causal relationship between maternal varicella and congenital abnormalities can be verified by detection of viral DNA or antigens in the infant. Contrary to congenital rubella or CMV infection, VZV has not been isolated in cell cultures from any infant with CVS. Serologic diagnosis is mostly based on the detection of VZV-specific IgG beyond 7 months of age, since IgM antibodies are present in about 25% of infants with CVS. Neonatal varicella is easily diagnosed on the basis of the typical clinical picture but PCR should be used for laboratory diagnosis from skin swabs or biopsies, CSF and tissue samples [42, 45].

112.4.3.5 Treatment

Antiviral therapy with acyclovir or valacylovir for intrauterine varicella should be started as soon as possible, preferably after contact with infected children. If a woman develops chicken pox at or within a few days of delivery, prophylactic treatment of infants with acyclovir may be appropriate. Neonates exposed to VZV should receive varicella-zoster immune globulin (VZIG). If neonates show signs of either infection, immediate treatment with acyclovir must be initiated. These newborns are at high risk for death due to chicken pox since they do not have maternally acquired antibodies to VZV [38, 45].

112.4.3.6 Prevention

A pregnant woman who does not recall having chicken pox or the vaccine and who is exposed in her household should promptly receive VZIG and tested for immunity, to prevent maternal infection or subsequent complications. Although no controlled studies show that VZIG prevents CVS, the biologic plausibility of this treatment comes from favorable results in improving or preventing clinical varicella in immunocompromised or otherwise healthy patients. However, the effectiveness of VZIG has not been evaluated later than 96 h [44].

Varicella pneumonia must be regarded as a medical emergency, since pregnant women are at risk of life-threatening ventilatory compromise and death. Neonatal varicella is more severe if maternal rash appears 5 days prior to or 2 days after delivery. The newborn should be given VZIG immediately. Intravenous acyclovir is recommended for maternal pneumonia and the severely affected neonate. No controlled study has yet evaluated the effectiveness of acyclovir or valacyclovir for postexposure prophylaxis to pregnant women or neonates. Most women who do not recall having had chicken pox are in fact immune, but delaying the administration of VZIG while awaiting a test result is inappropriate. Whereas VZIG is very effective in either preventing or reducing the severity of maternal disease, its effectiveness in preventing intrauterine infection is unknown; nonetheless VZIG is recommended for administration to women during pregnancy [43, 46].

Varicella vaccine, which is a live attenuated strain of varicella virus, is recommended for all adults who have not had chickenpox. Pregnant women should not be immunized because of the potential risk for fetal infection. Women inadvertently immunized 3 months before pregnancy or during pregnancy can be reassured about the low risk and the incident should be reported to the public health authorities and the vaccine manufacturer. If a pregnant woman, for whatever reason, received varicella vaccine in the first half of pregnancy, VZIG may be given [47, 48].

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113

Fetal infections: Rubella, HIV, HCV, HBV, and Human Parvovirus B19

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113.1 Rubella Virus

Rubella: Salient Points

- Congenital rubella (CR) infection leads to damage in over 80% of fetuses during the first trimester of pregnancy, in 25–34% of cases in the second trimester, while no malformations occur in the third trimester.
- CR syndrome (CRS) is characterized by the combination of cardiac, ocular, and hearing defects, although any organ may be affected leading to long-term disabilities.
- To prevent CRS it is essential to verify before or during pregnancy, that all women have natural or vaccine-induced immunity to the rubella virus.

113.1.1 Etiology and Pathogenesis

The importance of congenital rubella (CR) was recognized in the 1940s and the etiological agent was discovered in the 1960s. Rubella epidemics occur at 6- to 9-year intervals, and major pandemics every 10–30 years. Theoretically, universal immunization programs should eradicate rubella, but at least 100,000 cases of CRS still occur annually worldwide [1].

The rubella virus, a member of the togavirus family, is composed of an icosahedral nucleocapsid containing a single-stranded RNA genome, surrounded by a lipid envelope. The mechanism by which the virus causes fetal damage is poorly understood. The virus spreads through the bloodstream, it can infect and replicate in the placenta, it then reaches the fetus, where it persistently infects cells, inducing a decreased growth rate. In the first trimester of gestation the fetus is unable to mount an adequate immune response and the passive transfer of maternal antibodies is inefficient, thus the viral replication leads to altered organogenesis. From the second trimester of gestation, the risk of congenital rubella syndrome (CRS) decreases, in fact changes in the placenta, the appearance of the immune response, and the passive transfer of maternal IgG result in a higher protection from viral damage. The virus can persist for over a year in target organs, where it can undergo recurrent replications. Late onset CRS manifestations are due to persistent virus-driven tissue damage and scarring.

113.1.2 Transmission

Miscarriage, stillbirth and a series of birth defects (low birth weight, failure to thrive, congenital heart disease and central nervous system damage) are possible sequelae of CRS. Fetal infection may occur at any stage of pregnancy. The outcome of infection depends on the gestational age. Primary maternal infection causes damage in more than 80% of the fetuses during the first trimester of pregnancy, in 25–34% in the second trimester, while no malformations occur in the third trimester. Rubella reinfection may also occur both in vaccine-immune and, to a lesser extent, in naturally immune subjects. In these cases, the risk of CRS is estimated to be around 5% in the first trimester of pregnancy.

113.1.3 Clinical Aspects

Rubella infection spreads by droplets and it is usually subclinical or paucisymptomatic in infants and children. Clinical manifestations (fever, non-confluent maculopapular rash, headache, malaise, lymphoadenopathy, usually involving sub-occipital, post-auricolar and cervical nodes) develop after 14–21 days incubation.

CRS is typically characterized by the combination of cardiac, ocular, and hearing defects, although the virus can infect virtually any organ (Table 113.1) [2].

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 113.1 Congenital rubella syndrome main defects (listed in order of decreasing frequency)

Early onset manifestations
- Deafness
- Mental retardation
- Cardiovascular defects affecting primarily the left atrium and the heart septa
- Ocular defects (nuclear cataracts, microphthalmia, pigmentary retinopathy, glaucoma)
- Thrombocytopenia
- Hepatitis
- Bone lesions
- Dental defects
- Hypospadias
- Cryptorchidism
- Inguinal hernia
- Interstitial pneumonitis
- Meningo-encephalitis
- Cerebral calcification
- Splenic fibrosis
- Nephrosclerosis
- Nephrocalcinosis

Late onset manifestations
The reported delayed manifestations (due to the altered immune system of CRS patients) underscores the importance of careful follow-up of these patients
- Insulin-dependent diabetes
- Thyroid dysfunction
- Neurodegenerative disorder (panencephalitis)
- Slower growth rate during preschool years
- Smaller head circumference
- Ocular defects (secondary glaucoma, strabismus)
- Congenital defects in the immune system (transitory hypogammaglobulinemia, especially ipoIgA, defects in cell-mediated immunity)

113.1.4 Diagnosis

Amniotic fluid and fetal blood samples have been used to detect fetal rubella infection by means of nested reverse-transcriptase polymerase chain reaction (RT-PCR). Fetal blood, obtained by cordonocentesis, can also be tested for Rubella IgM. Amniocentesis is less invasive than fetal blood sampling. Both tests should be performed 6–8 weeks after maternal infection; the best reliability is when the fetus is 22 weeks old.

Postnatally, CRS is diagnosed by detection of rubella-specific IgM, though both false negative and false positive results have been described, or by stable or increased titers of specific IgG over several months. IgG avidity testing may help to differentiate between recent and past infection [3]. Identification of the virus in urine, blood or nasopharyngeal secretions by cultures or by RT-PCR may also confirm the diagnosis.

113.1.5 Prognosis

Children affected by CRS may require medical, surgical, educational and rehabilitative management. CRS must be managed as a dynamic rather than a static disease, requiring a multidisciplinary approach: for instance, early treatment is critical for hearing defects and psychomotor difficulties.

113.1.6 Therapy and Treatments

The prime strategy for the prevention of CRS is to ensure that all pregnant women are immune to rubella. In developed countries, vaccination programs are based on the universal immunization of infants at 12–15 months of age, plus a second vaccination in adolescents to achieve immunity in patients with primary vaccine failure. Furthermore, all women at childbearing age should be tested and targeted vaccinated, if still susceptible. The RA 27/3 strain is used as a rubella vaccine. This is remarkably safe, has high efficacy (over 90%), and induces long-lasting protection. It is used as a component of the measles-mumps-rubella (MMR) vaccine or of the recent tetravalent vaccine (MMR plus Varicella).

Vaccination programs have led to a significant reduction in morbidity and mortality associated with CRS. Benefits of the vaccine far outweigh the costs of treatment and rehabilitation of affected children and adults.

113.2 Human Immunodeficiency Virus (HIV)

HIV: Salient Points
- Mother-to-child transmission (MTCT) is the most common route of HIV infection in children.
- MTCT may occur in utero and at the time of delivery. Breast-feeding is another route of transmission.
- The MTCT rate is estimated to be 15–20% in bottle-fed infants, but the implementation of preventive strategies has reduced the risk to approximately 1–2%.
- Effective preventive interventions include the administration of prenatal antiretroviral (ARV) prophylaxis, elective cesarean section, administration of ARV during delivery and for the infant, avoidance of breastfeeding.
- The early diagnosis of infection in exposed infants is based on detection of HIV-RNA by PCR assays in two separate determinations.
- An early combined ARV therapy in HIV infected infants offers the great benefits of a longer asymptomatic period and of a prolonged survival.

113.2.1 Etiology and Pathogenesis

More than 2.5 million children are estimated to be living with human immunodeficiency virus type 1 (HIV-1) infection worldwide, mostly following mother-to-child transmission (MTCT).

HIV is a retrovirus belonging to the subgroup of lentiviruses. The first step of its life cycle is characterized by the integration of the virion envelope glycoproteins (gp120 and gp41) with the cell surface CD4 molecule. After entering the cell, the virus is rapidly uncoated. Then, through reverse transcriptase its viral RNA is transformed into linear DNA. This is circularized and transferred into the nucleus where it is inserted at random sites as a provirus. The provirus is activated by host cell responses to antigens, cytokines or products of other viruses. Thousands of infectious particles can originate from a single infected cell, either chronically, over weeks, or as a single burst resulting in cell death. HIV infection leads to a profound qualitative and quantitative attrition of the immune system, particularly of cell-mediated immunity with a decrease in CD4+ cell count. Immune mediated damage presumably plays a key role in AIDS pathogenesis.

Two genetically distinct HIV types have been identified: HIV-1 and HIV-2; the latter is localized mainly in Western Africa and causes a less aggressive disease.

HIV infection results in a minimal risk of adverse pregnancy outcomes, such as spontaneous abortion, intrauterine growth restriction, and premature delivery [4].

113.2.2 Transmission

HIV is a sexually transmitted infection that also spreads by contact with infected blood. After the screening of blood donors, MTCT has become the most common route of infection in children. MTCT ranges between 15–23% in bottle-fed infants. Prolonged breastfeeding may account for an additional 10–15% of infections. With the implementation of effective, cost saving preventive measures, MTCT rate can be reduced to 1–2% in non breast-fed infants.

Viral, maternal, obstetrical, fetal and infant factors influence the risk of MTCT (Table 113.2). Most children are infected perinatally, though intrauterine transmission may occur. An infant is considered to have been infected in utero when HIV-1 can be detected from peripheral blood within 48 hours from birth. In contrast, a non breast-fed child is taken to have had intrapartum infection if he/she becomes viremic subsequently. Transmission due to household contacts is not documented.

113.2.3 Clinical Manifestations

According to the infection status, an infant born to a seropositive mother is classified as exposed (E), infected (I) or seroreverted (SR) (see below). A breastfed infant is at risk of infection in the 6 months after interruption of maternal lactation.

HIV+ children are classified into mutually exclusive, progressive categories according to their clinical and immunological status (Table 113.3) [5]. Once classified in a more severe category a child cannot be reclassified in a less severe one, even if the clinical or immunologic situation improves.

HIV disease progression is more accelerated in children than in adults. HIV-infected newborns are usually asymptomatic, but they can become seriously ill within the first weeks to months of life. Growth delay is an early finding in untreated perinatally infected infants. Children are more likely than adults to have serious bacterial infections and lymphoid interstitial pneumonia is almost entirely restricted to the pediatric age. *Pneumocystis jirovecii* pneumonia (PCP) is the most common serious opportunistic infections (OI) in infected infants, with the highest incidence at 3–6 months of age.

113.2.4 Diagnosis

Diagnosis of HIV infection in exposed infants is based on the detection of viremia in two separate determinations. Most uninfected children lose passively acquired maternal antibodies by 12-15 months of age, though these may persist in 2% up to 18 months. Criteria for diagnosis or exclusion of infection [6] through detection of viremia are detailed in Table 113.4.

Table 113.2 Maternal, obstetrical, and neonatal factors associated with increased HIV transmission rate

Pregnancy	Labour and delivery	Breastfeeding
• High maternal viral load (new infection or advanced AIDS) and/or CD4 cell count < 200/mm^3 • Viral, bacterial, or parasitic placental infections, such as malaria • Sexually transmitted infections	• High maternal viral load (new infection or advanced AIDS) and/or CD4 cell count < 200/mm^3 at delivery • Rupture of membranes (> 4 hours) • Invasive delivery procedures that increase contact with the mother's infected blood or body fluids (e.g., episiotomy, artificial rupture of membranes) • Chorioamnionitis (from untreated STI or other infections) • Preterm delivery	• High maternal viral load (new infection or advanced AIDS) and/or CD4 cell count < 200/mm^3 • Duration of breastfeeding • Breast abscesses, mastitis, nipple fissures • Oral disease in the baby (e.g., thrush or sores)

Table 113.3 Classification system for HIV infection in children younger than 13 years of age

	Clinical categories			
Immune categories (Based on age-specific CD4+ cell count or percentage)	No symptoms (N)	Mild symptoms (A)	Moderate symptoms (B)	Severe symptoms (C)
1 - No suppression 0–11 months → > 1500 cells/μL (≥25%) 1–5 years → > 1000 cells/μL (≥25%) > 6 years → >500 cells/μL (≥25%)	N1	A1	B1	C1
2 - Moderate suppression 0–11 months → > 750-1499 cells/μL (15-24%) 1–5 years → 500-999 cells/μL (15-24%) > 6 years → 200-499 cells/μL (15-24%)	N2	A2	B2	C2
3 - Severe suppression 0–11 months → > < 750 cells/μL (<15%) 1–5 years → > < 500 cells/μL (<15%) > 6 years → < 200 cells/μL (<15%)	N3	A3	B3	C3

Modified from [5].

Table 113.4 Diagnosis of HIV infection in exposed children less than 18 months of age [6]

Diagnosis of HIV Infection (I)	Exclusion of HIV-1 infection (SR)
1. Positive results on two separate specimens (not including cord blood) from one or more of the following tests: – HIV nucleic acid detection and/or – HIV p24 antigen (for a child > 1 month) and/or – HIV isolation (viral culture) 2. Severe clinical conditions resulting from HIV-1 infection	1. At least two negative HIV-1 RNA or DNA virologic test results from separate specimens, both of which obtained at > 1 month of age (1 of them after 4 months of age) 2. At least two negative HIV-1 antibody test results from separate specimens obtained at > 6 months of age and no other laboratory or clinical evidence of HIV-1 infection

HIV nucleic acid detection is the method of choice for the diagnosis or exclusion of infection in children < 18 months of age. Although HIV culture can be used, it is less standardized and less sensitive than nucleic acid detection tests. The use of p24 antigen testing to exclude infection in children <18 months is not recommended because of poor sensitivity, especially in presence of HIV antibody. Quantitative RNA tests have been approved for monitoring HIV infection and qualitative RNA tests have been approved to aid diagnosis. HIV-2 can be diagnosed with HIV-2 DNA PCR.

113.2.5 Prevention of MTCT

All pregnant women should be tested routinely for HIV during an early prenatal visit (first trimester) in order to adopt effective preventive strategies. These include

- administration of maternal antiretroviral (ARV) prophylaxis during pregnancy and at delivery
- elective cesarean section
- ARV treatment of the infant
- avoidance of breastfeeding.

113.2.5.1 Prenatal ARV Prophylaxis

Prenatal ARV regimens with highly active antiretroviral therapy (HAART) from 28 gestational weeks, regardless of viral load and CD4 cell count, are recommended to prevent MTCT [7]. In particular, women undergoing ARV treatment should continue their therapy during pregnancy, while those who do not require treatment for their own health should receive a three-drug combination to prevent HIV transmission to their offspring. Zidovudine (ZDV) prophylaxis alone is controversial, but it may be considered when maternal HIV RNA level is < 1000 copies/mL. After the onset of labor or rupture of membranes (or approximately 3 hours before an elective cesarean section) intravenous ZVD (2 mg/kg over the first hour, then 1 mg/kg per hour until delivery is complete) should be administered to all infected women, even if they are under HAART and their viral load is undetectable.

Although animal studies suggest possible congenital abnormalities with some antiretroviral drugs, results from registries and cohort studies do not confirm this association in human beings.

Mitochondrial toxicity of clinical relevance has been described, albeit anecdotically, in Europe after in utero exposure to nucleoside analogues, but these findings are still a matter of debate. There is no evidence that exposure to antiretroviral drugs in utero or neonatally is associated with an increased risk of childhood cancer.

Table 113.5 Zidovudine prophylaxis in HIV exposed infants [8]

Gestational age at birth	Oral dose (mg/kg per dose)	Intravenous dose (mg/kg per dose)	Frequency	Weeks
≥ 35 weeks	2	1.5	Every 6 hours	6
30 > weeks < 35	2	1.5	Every 12 hours advancing to every 8 hours at 2 weeks of age	6
< 30 weeks	2	1.5	Every 12 hours advancing to every 8 hours at 4 weeks of age	6

113.2.5.2 Elective Cesarean Section

The MTCT rate is lower in women who delivered by elective cesarean section than in those who delivered by emergency cesarean section or vaginal delivery [7]. There is general agreement that elective C-section should be recommended at 38 weeks' gestation to all pregnant women with a viral load > 1000 RNA copies per mL near the time of delivery [8]. The benefit of elective C-section in women with low or undetectable viral loads and/or under HAART therapy remains questionable, though it is widely adopted in some countries.

113.2.5.3 Infant ARV Prophylaxis

A 6 week course of ZDV prophylaxis is recommended for all exposed neonates and should be initiated as soon as possible after birth (Table 113.5). In certain situations (e.g., treated women with suboptimal viral suppression, or only intrapartum ARV therapy or known ZDV-resistant virus) some experts suggest adding other ARV drugs [9].

113.2.5.4 Breast-feeding

Although the risk of postnatal HIV transmission through maternal milk is well documented, the WHO recommends breastfeeding in the first 6 months of life in some developing countries, because such a risk is lower than that of death from diarrheal diseases and malnutrition. Conversely, in countries where replacement formula feeding is acceptable, feasible and safe, breastfeeding should be discouraged [10].

113.2.6 Prognosis

The prognosis of perinatally acquired HIV infection was initially poor, particularly for infants with in utero infection or early clinical manifestations. Opportunistic infections (OIs), such as PCP, progressive neurological disorders, malignancies, severe bacterial infections, and wasting syndrome were the main causes of death. Morbidity and mortality have significantly decreased since the introduction of HAART in

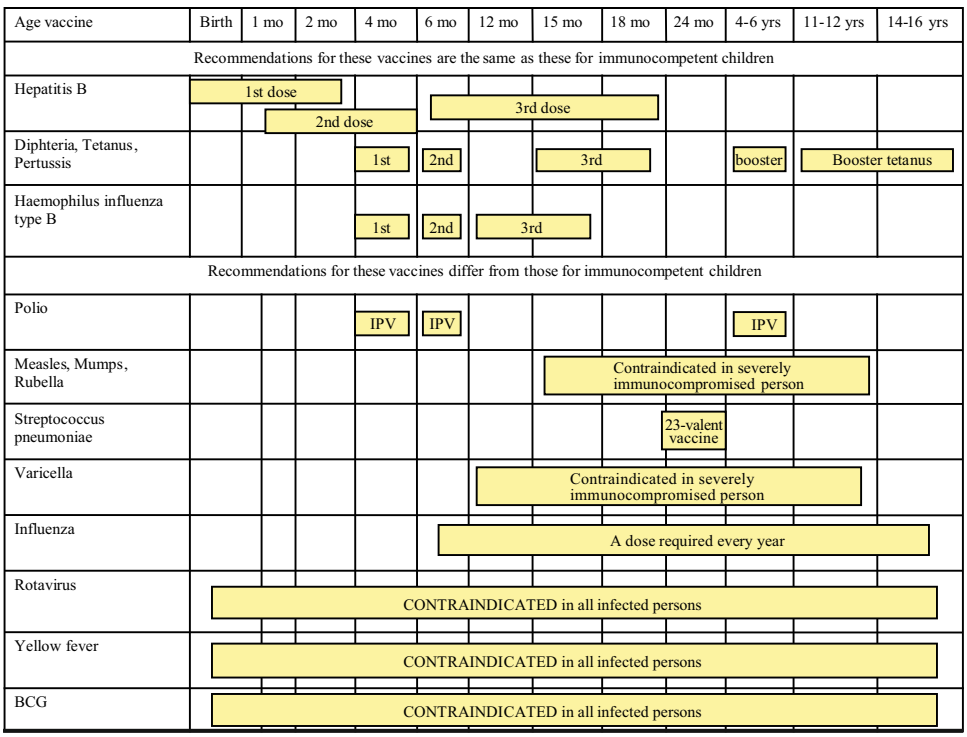

Fig. 113.1 Recommendations for routine immunizations in HIV infected children. Modified from [12]

Table 113.6 Prophylaxis to prevent *Pneumocystis jirovecii* pneumonia in HIV-exposed infants or HIV-infected children

Pathogen	Indication	First choice	Alternative
Pneumocystis jirovecii	Exposed children: – from 6 weeks until exclusion of infection Infected children: – from 6 weeks until 1 year – from 1-5 yrs if CD4 < 500 cells/mm^3 – from 6-12 yrs if CD4 < 200 cells/mm^3 or percentage < 15%	TMP-SMX 150/750 mg/m^2/die orally divided in 2 doses daily and administered 3 times weekly on consecutive days	– Dapsone (>1 mos of age) 2 mg/kg orally daily or 4 mg/kg weekly – Atovaquone: children aged 1–3 mos and >24 mos, 30 mg/kg orally daily; children aged 4–24 mos, 45 mg/kg orally daily – Aerosolized pentamidine: >5 yrs, 300 mg every months

MTCT mother to child transmission, *TMP* trimethoprim, *SMX* sulfamethoxazole, *TST* tubercolin skin test, *mos* months, *yrs* years. Modified from [12].

children with good treatment adherence. Most of these children remain in good health for many years.

113.2.7 Therapy and Treatments

113.2.7.1 Antiretroviral Therapy

Initiation of ARV therapy is recommended for all infected infants < 12 months at the time of diagnosis, regardless of clinical condition and immunological parameters. Subsequently, the therapeutic options should be based on clinical, immunologic and virologic findings. The initial regimen should include two nucleoside reverse transcriptase inhibitors (NRTIs) plus a highly active protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) as the initial regimen [11]

113.2.7.2 Supportive Care

All HIV-infected children should be protected from vaccine-preventable diseases (Fig. 113.1). In general, all inactivated vaccines can be administered safely, and usual doses and schedules are recommended. Children with HIV infection are at a high risk for complications of varicellazoster and measles. Bearing in mind the limited safety, immunogenity and efficacy of varicella and MMR vaccines in HIV infected children, these should be considered for subjects who are not severely immunosuppressed (CD4+ cell percentage > 15%).

Prophylactic measurements to prevent PCP pneumonia are detailed in Table 113.6 [12].

113.3 Hepatitis C Virus (HCV)

HCV: Salient Points

- Mother to child transmission (MTCT) is the most common route of HCV infection in the pediatric population.
- Maternal HCV infection does not impair the course of pregnancy or increase the risk of spontaneous abortion or fetal death.
- No interventions are currently available to prevent MTCT of HCV.
- Early diagnosis of infection in exposed infants relies on the detection of HCV-RNA by PCR.
- The natural history of vertically acquired HCV infection in infancy and childhood is relatively benign.

113.3.1 Etiology and Pathogenesis

The hepatitis C virus (HCV) is the most common cause of chronic liver disease, with 130 million infected individuals worldwide and about 3 million new infections each year. The prevalence of the infection varies from 0.5–2% in Western World to 5–15% in some endemic African areas. After the screening of blood donors with consequent disappearance of post-transfusional hepatitis C, MTCT has become the most common route of infection in the pediatric population.

HCV is a small (50 nm in size), enveloped, single-stranded RNA virus. Six major genotypes exist; each genotype responds differently to antiviral therapies and comprises hundreds to thousands of subtypes, referred to as quasi-species, resulting from the high viral mutation rates and reflecting the unique ability of the virus to continually alter its immunologically recognizable epitopes. HCV primarily enters hepatocytes, although other cell types, such as dendritic cells and B-cells, can also be infected. The virus is non-cytopathic by itself.

113.3.2 Transmission

Because of the absence of effective preventive strategies, routine screening for HCV in pregnant women is not recommended. However, screening is suggested in special circumstances (women positive for HIV or hepatitis B virus, a history of intravenous drug use, transplantation, hemodialysis, blood, or blood products transfusion prior to 1992, tattooing or unexplained elevated liver enzymes). In this setting,

antenatal screening might be useful to identify infected children before the onset of symptoms and to offer post-pregnancy therapy to their mothers.

HCV infection does not seem to impair the course of pregnancy or increase the risk of spontaneous abortion or fetal death. The time of transmission remains unclear. The largest studies consistently estimate that 30–50% of HCV infections are acquired in utero while the remainder occur in the late intrauterine/intrapartum period [13].

Several risk factors for MTCT have been identified (Table 113.7) [14]. However, none are modifiable and no preventive interventions can be adopted.

High viral load is associated with MTCT. Thus, infected women with undetectable HCV-RNA during pregnancy can be reassured that the risk of transmission to their infants is very low, though it cannot be excluded. Anti-viral drugs, such as interferon-alpha plus ribavirin cannot be used in pregnancy to reduce viremia due to their teratogenic effects. Viral clearance prior to pregnancy would increase the likelihood that a woman remains non-viremic in pregnancy. In this context, women should be advised not to conceive until at least 6 months after cessation of therapy.

Although the evidence is inconclusive, it is prudent to avoid amniocentesis, instrumental vaginal delivery and a prolonged rupture of membranes. These procedures should only be used when indicated to avoid maternal or infant morbidity. Elective cesarean section should not be offered to HCV infected women to prevent transmission and breastfeeding should not be discouraged.

Table 113.7 Potential risk factors for HCV mother-to-child transmission

Variable	Risk factor	Strength of the evidence
Maternal HCV/HIV coinfection	Yes	Strong
High maternal viral load	Yes	Sufficient
Cesarean section	No	Sufficient
Vaginal delivery	No	Sufficient
Breast feeding	No	Sufficient
Girls gender	Yes	Sufficient
Amniocentesis	?	Insufficient
IV drug users	?	Insufficient
ALT levels during pregnancy	Yes	Insufficient
Internal fetal monitoring	Yes	Insufficient
Forceps deliveries	Yes	Insufficient
Perineal or vaginal lacerations	Yes	Insufficient
Episiotomy	No	Insufficient
Prolonged membrane rupture	Yes	Insufficient
Cigarette smoking	No	Insufficient
Alcohol intake	No	Insufficient
Maternal age	No	Insufficient
HCV genotype	No	Insufficient
Numbers of pregnancies	No	Insufficient
Prematurity	No	Insufficient
Genetic background	Yes	Insufficient

113.3.3 Clinical Aspects

The natural history of vertically acquired HCV infection remains to be elucidated.

Infected children are asymptomatic at birth and their general condition usually remains good in the first decade of life with regular growth. Only a fraction develop minor abnormalities, such as hepatomegaly, or non specific symptoms and signs.

Alanine aminotransferase (ALT) activity is highest in the first two years of life, after which it declines, often assuming a fluctuating pattern. Viremia also fluctuates in a large proportion of infected children.

Liver function tests are of little help in assessing the development of aggressive hepatitis and cirrhosis. Necrotic inflammation and fibrosis are usually milder in children than in adults. Progression of liver damage is not linear and cirrhosis or HCC are rare in childhood. Routine liver biopsy in children with HCV is not recommended.

Extrahepatic manifestations, such as mixed cryoglobulinemia and autoimmune disorders, are rare in childhood. However, autoantibodies are frequently detected in infected children [15].

113.3.3.1 Diagnosis

Persistence of anti-HCV antibody beyond 18 months of age represents the "gold standard" for the diagnosis of vertically acquired HCV infection. Earlier diagnosis in an exposed child relies on the detection of HCV-RNA by PCR. Children are taken to be infected if PCR positive in at least two separate determinations. The test is highly specific, but its sensitivity is low at birth (22–33%), whereas it increases up to 85% after the first month of life [16].

It must be underlined that a negative PCR might also reflect fluctuations in viremia. Thus, both antibody testing and the detection of serum HCV RNA are recommended [17] for definition of the infection status in exposed children (Fig. 113.2).

113.3.4 Therapy and Treatments

Limited data are available for the management of children and adolescents with chronic HCV infection [18]. Treatment algorithms often are based on adult trials. Treatment is offered to selected patients (Table 113.8). Since histological progression is rare within the first three years of life and treatment may give rise to significant side effects, such as the interferon-induced growth impairment, this should be delayed to older children [19].

Children with HCV should be immunized against hepatitis A and B.

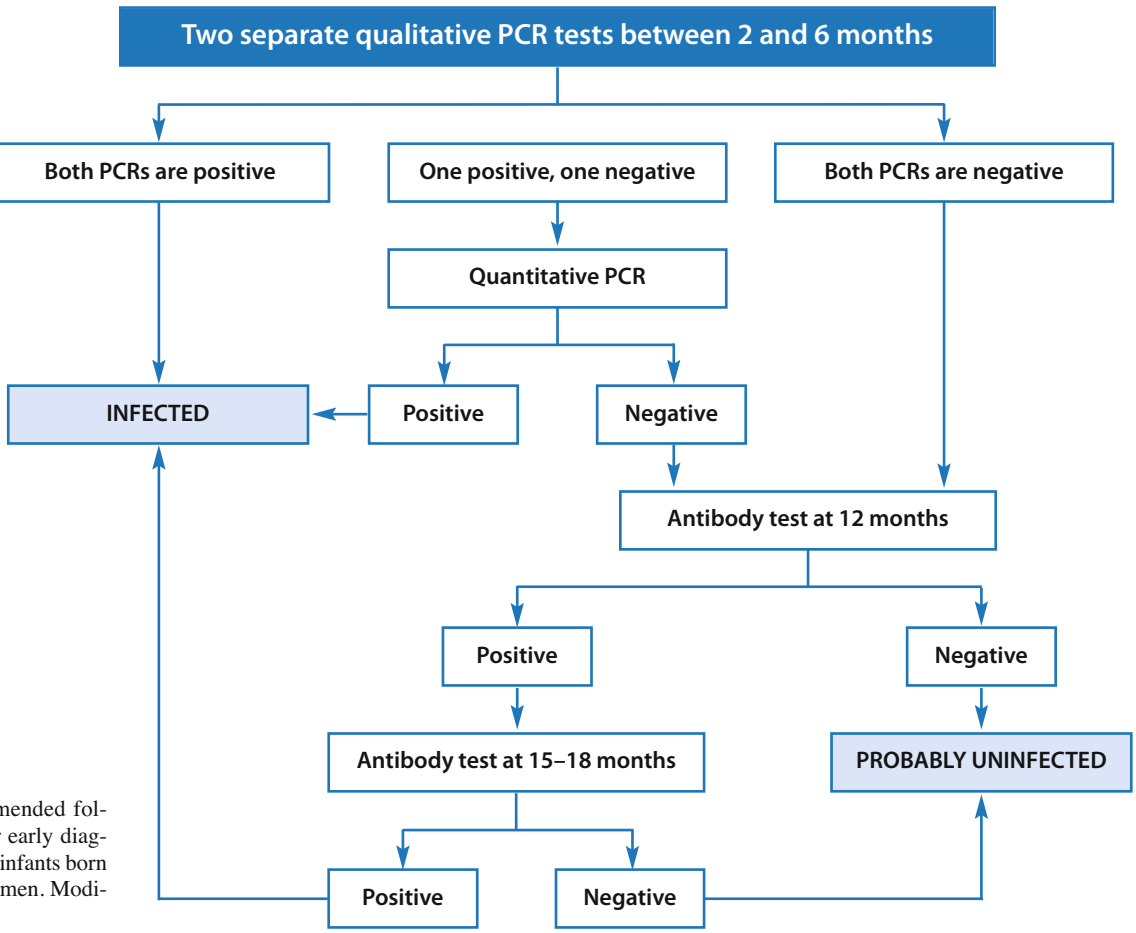

Fig. 113.2 Recommended follow-up schedule for early diagnosis of infection in infants born to HCV infected women. Modified from [13]

Table 113.8 Treatment for chronic HCV infection

Drugs	Dosage	Duration of therapy
IFN-α*	3-5 million units per square meter of body surface, intramusculary or subcutaneously, three times a week for 6-12 months	• 48 weeks for genotype 1 • 24 weeks for genotype 2 or 3
PEG-IFN-2αb**	60 mcg/m^2/week injected subcutaneously once a week	
Ribavirin	15 mg/kg/day orally in two divided doses (capsule and oral solution)	

* Recombinant IFN-alpha.
** Pegylated interferon alpha-2b recently approved by the U.S. Food and Drug Administration for the treatment of chronic hepatitis C virus infection in previously untreated children and adolescents 3 years or older [25].

113.4 Hepatitis B Virus (HBV)

HBV: Salient Points

- Mother to child transmission (MTCT) is the main route of HBV infection in children.
- More than 90% of perinatally exposed children develop chronic infection.
- All term infants born to HbsAg-positive women should receive HBV vaccine and specific immunoglobulins at birth, followed by completion of three-dose vaccine series.
- Universal infant immunization has reduced the MTCT rate by 85–90%.

113.4.1 Etiology and Pathogenesis

Two billion people worldwide are estimated to have acquired hepatitis B virus (HBV), and more than 350 million to be chronically infected [20]. In low prevalence areas (chronic infection < 2%), such as the USA and Western Europe, most

new infections occur in young adults and are acquired sexually or through intravenous drug use, while in moderate or high prevalence areas (chronic infection 2–7% or > 8%, respectively) perinatal and horizontal transmission are the prevalent routes of infection.

HBV is a member of the Hepadnavirus family. The virus is divided into four major serotypes (adr, adw, ayr, ayw) and into eight genotypes (A-H). Differences between genotypes affect the disease progression and the response to treatment.

Hepatocytes are the primary target of HBV, but the kidney, pancreas and mononuclear cells can also be infected. HBV is non-cytopathic by itself. The host's immune response causes both viral clearance and hepatocellular injury. The cytotoxic T-cell-mediated lysis of infected hepatocytes is the predominant mechanism of liver damage. Intrauterine exposure to HBV antigens may induce tolerance, accounting for the high rate of carriers among perinatally infected children.

113.4.2 Transmission

Possible routes of HBV transmission include unprotected sexual contact, blood transfusions, re-use of contaminated needles or syringes, and MTCT. The vertical transmission rate is 70–90% in untreated infants of HB surface antigen (HbsAg) and HBe antigen (HbeAg) positive viremic women, while it decreases to 10–30% in the HBsAg carrier mothers who have anti-e antibody (anti HBe) and are PCR negative. In utero infection is uncommon and mainly takes place during acute maternal infection. Transmission to the offspring mostly occurs at delivery by microtransfusion or contact with contaminated body fluid. The mode of delivery (cesarean *vs* vaginal) does not affect transmission, and breastfeeding is not an additional risk factor in infants treated with proper immunoprophylaxis.

Acute HBV hepatitis or exacerbation of chronic disease may occur during pregnancy. However, these conditions increase neither maternal morbidity or mortality nor the risk of fetal complications, though a high number of preterm labors have been reported in women with acute hepatitis B.

113.4.3 Clinical Aspects

The age of HBV acquisition is roughly inversely correlated with the likelihood of developing a persistent infection. Neonates have > 90% risk of developing chronic infection, as compared to 25–50% of older children and adolescents and 5% of adult subjects.

113.4.3.1 Acute Infection

HBV infected infants and children are usually asymptomatic. However, disease onset may be insidious. Symptoms and signs include anorexia, malaise, nausea, vomiting, abdominal pain and jaundice.

Extrahepatic manifestations encompass skin rashes, membranous glomerulonephritis, arthralgias, and arthritis. MTCT is the most important route of transmission for acute or fulminating hepatitis in infancy. The incubation period ranges from 6 weeks to 6 months and the mortality rate of fulminating hepatitis is high (around 67%) and may often require liver transplantation.

113.4.3.2 Chronic Infection

Chronic infection is defined as a persistence of HBsAg for > 6 months. Affected children are usually asymptomatic with normal growth. In vertically infected children, the spontaneous clearance of HBV (i.e., loss of HBsAg and development of anti-HBs Antibody) occurs at a rate of 0.6% per year over the first decade of life.

Most children who remain infected develop immune tolerance and have normal levels of hepatic transaminases, which usually mirror less active hepatic inflammation. Chronic hepatitis B may exhibit various degrees of liver inflammation and fibrosis on biopsy. The extent of inflammation and fibrosis or cirrhosis correlates with a worse prognosis. Less then 4% of infected children have persistent viral replication (HBeAg+) and elevated HBV DNA levels with ongoing inflammatory hepatitis and persistently or intermittently elevated transaminase levels (active hepatitis). Most concerns are focused on the latter patients, because they could develop cirrhosis and hepatocarcinoma (HCC) over a 20–30 year period.

113.4.3.3 Diagnosis

All pregnant women should be tested routinely for HBsAg during an early prenatal visit (first trimester) at each pregnancy, including women previously vaccinated or tested.

Antigens and antibodies to be tested include: HBsAg and specific antibody (anti-HBs), hepatitis B core antigen (HBcAg) and specific antibody (anti-HBc), HBeAg and anti-HBe, bearing in mind that at least one serologic marker is present during the various phases of infection (Table 113.9). The presence of a confirmed HBsAg is indicative of ongoing infection. The detection of HBeAg and HBV DNA-positive PCR document viral replication. In general, these markers correlate with high infectivity. The appearance of anti-HBe means a loss of replicating virus, although reversion to HBeAg positivity may occur.

In exposed infants who undergo proper immunoprophylaxis, HBsAg and anti-HBs should be checked at 9–15 months of age or 1–3 months following completion of the primary immunization series to assess whether they are immune or infected.

Table 113.9 Interpretation of serologic tests result for hepatitis B virus infection

Serologic markers				Interpretation
HBsAg	Total anti-HBc	IgM anti-HBc	Anti HBs	
–	–	–	–	Never infected
+	–	–	–	Early acute infection; transient (up to 18 days) after vaccination
+	+	+	–	Acute infection
–	+	+	–	Acute resolving infection
–	+	–	+	Recovered from past infection and immune
+	+	–	–	Chronic infection
–	+	–	–	False positive (i.e., susceptible); past infection; "low-level" chronic infection; passive transfer to infants born to HBsAg-positive mothers
-	-	-	+	Immune if concentration is > 10 mIU/mL; passive transfer after hepatitis B immune globulin administration

HBsAg hepatitis B surface antigen, *anti-HBc* antibody to HBV core antigen, *anti HBs* antibody to surface antigen.

113.4.4 Prevention

All term infants born to HBsAg+ women should receive hepatitis B vaccine and hepatitis B immunoglobulin (HBIG) 12–24 hours after birth, administered at different injection sites, followed by completion of the three-dose vaccine series. This schedule prevents infection by 85–95%. Transplacental transmission cannot be interrupted by immunoprophylaxis and may account for the small percentage of unprotected infants.

Hepatitis B immunoprophylaxis for preterm infants differs slightly (Table 113.10).

113.4.5 Therapy and Treatments

Therapy for HBV infection has not been studied in neonates. Antiviral treatment is contraindicated in children under 3 years of age, given the adverse effects of interferon administered early in life. In general, children with normal ALT levels are not candidates for antiviral treatment.

113.4.5.1 Acute HBV Infection

There is no treatment of proven efficacy for children with acute HBV infection.

113.4.5.2 Chronic HBV Infection

General management of children with chronic infection includes [21]:

- education and counseling of family members (including vaccination with hepatitis B vaccine)
- periodic clinical checks and laboratory investigations for liver disease

Table 113.10 Hepatitis B immunization of term and preterm (< 2000 g) infants by maternal hepatitis B surface (HBsAg) status

Newborn	Maternal HBsAg status	Management		
		Drugs	Dose	Age
Mature infants and preterm infants weighing > 2000 g	Positive	Vaccine	1	Birth (< 12 hours)
		Vaccine	2	1-2 months
		Vaccine	3	6 months
		HBIG	0.5 mL intramusculary	Birth (< 12 hrs)
	Unknown	Vaccine	1	Birth (< 12 hrs)
		Vaccine	2	1-2 months
		Vaccine	3	6 months
		HBIG*	0.5 mL intramuscularly	< 1 week of age**
Preterm infants weighing ≤ 2000 g	Positive	Vaccine	1	Birth (< 12 hrs)
		Vaccine	2	1 month
		Vaccine	3	2-3 months
		Vaccine	4	6-7 months
		HBIG	0.5 mL intramuscularly	
	Unknown	As infants born to HbsAg positive mother		
	Negative	Delay first dose of vaccine until 1 month of age or hospital discharge. Complete the vaccine series		

* If mother tested as soon as possible after admission for delivery is found to be HBsAg positive.
** As soon as possible if HBsAg positive status is confirmed.

- vaccination with hepatitis A vaccine
- antiviral therapy, if appropriate

No limitations in terms of school or other activities should be placed in children with chronic HBV infection.

The goals of chronic hepatitis B treatment are the cessation or reduction of viral replication, normalization of aminotransferase levels, and the prevention of cirrhosis, hepatic failure, and HCC.

Treatment with two antiviral drugs, i.e., interferon (IFN)-α and lamivudine are recommended for children over 3 years of age with evidence of chronic infection (i.e., detectable HBsAg for at least 6 months), active viral replication (i.e., the presence of HBeAg and/or elevated HBV DNA levels), and elevated ALT levels (Table 113.11). HBV DNA is also essential to monitor response to antiviral therapy. Elevated levels (> 20,000 IU/mL or > 10^5 copies/mL) after 24 weeks of therapy raise the highest concerns for liver health [22].

113.5 Human Parvovirus B19

Human Parvovirus B19: Salient Points

- 50% pregnant women are susceptible to B19 infection. The vertical transmission rate, in the first or second trimester, is around 30%.
- Primary maternal infection with parvovirus B19 is associated with asymptomatic fetal infection, nonimmune hydrops fetalis, intrauterine fetal death, and birth defects.
- There is no specific therapy against B19 infection; in case of hydrops and/or signs of fetal anemia, intrauterine erythrocyte transfusions are recommended.

113.5.1 Etiology and Pathogenesis

Parvovirus B19 (B19) is the only member of the family *Parvoviridae* to be pathogenic in humans. It may cause a wide spectrum of clinical manifestations; its seroprevalence increases with age and infection confers lifelong immunity.

B19 is a single-stranded non-enveloped DNA virus, genetically stable, resistant to heat and detergent inactivation, which can survive in blood products despite elimination procedures. It requires a mitotically active host cell for replication, such as erythroid precursors, fetal liver cells, or cord blood mononuclear cells. B19 infects these cells lytically. It uses at least three cellular receptors for cell attachment and entry. One of these, the glycolipid globoside (P-ag), is present on the hematopoietic precursors, endothelial cells, fetal myocytes, and placental trophoblasts. The presence of P-ag on these tissues may explain the hematological disorders, the myocardial disease, the congenital infection and the vasculitis syndromes.

113.5.2 Transmission

Parvovirus B19 is transmitted by respiratory route, blood products and vertically, from the mother to the offspring. About 50% of pregnant women are susceptible to B19 infection. In the first and second trimester of pregnancy the vertical transmission rate reaches 30%. Among vertically infected children, 5–10% presents an abnormal outcome. This risk is higher when maternal infection occurs in the first 20 weeks of pregnancy.

113.5.3 Clinical Aspects

Human parvovirus B19 infection may give rise to a large array of clinical manifestations, depending on the patient's immunologic and hematologic status. In the normal host, the infection may be asymptomatic or give rise to erythema infectiosum (EI) and/or arthropathy. EI, also referred to as Fifth disease or Slapped cheek syndrome, usually affects

Table 113.11 Treatment for chronic HBV infection

Drugs	Advantage	Disadvantage	Response in children
IFN-α [a]	No drug resistance Short duration of treatment	Parenteral administration Adverse effects common	20–58% HBV DNA or HBeAg loss [b]
PEG-IFN-2αa [c]	Administration once a week	Parenteral administration Adverse effects common	HBV DNA disappearance in 6 out of 13 children without any side effect [c]
Lamivudine [d]	Minimal adverse effects Oral administration Liquid formulation available	Drug resistance common (20%/years)	23–35% HBV DNA and HBeAg loss [e]

[a] Dosage in children > 3 years of age: 5-10 MU/m^2 three times a week for 24 weeks.
[b] From [27].
[c] In children there is only one preliminary report evaluating the rapid viral response of PEG-INF (100 μg/m^2/week) [26]. Not currently approved in chronic HBV infection in children.
[d] Dosage in children > 3 years of age: 3 mg/kg/day up to 100 mg/day for 52 weeks.
[e] From [28].

Table 113.12 Disorders associated with Parvovirus B19 infection

Autoimmune disorders
- Systemic lupus erythematosus
- Systemic vasculitides
- Rheumatoid arthritis
- Production of auto-antibodies to double-strained DNA, anti-nuclear soluble antigens, cardiolipin and rheumatoid factor

Increased bone marrow cell turnover
- Transient aplastic crisis (TAC) with severe anemia, resulting in congestive heart failure, cerebrovascular events and acute splenic sequestration
- Thrombocytopenia
- Neutropenia
- Pancytopenia

B19 persistence in immunocompetent patients
- Persistent infection with potentially severe and chronic anemia, with various long-lasting symptoms such as fatigue, fever, arthralgia and myalgia

Other associated disorders
- Myocarditis and heart failure
- Hepatitis
- Kawasaki disease
- Gloves-and-socks syndrome
- Neurological disease
- Fibromyalgia

Infection in immunocompromised individuals
- Persistent BM suppression with chronic anemia (predisposing conditions are congenital immunodeficiencies, leukemia, lymphoma, myelodysplastic syndrome, BM and solid organ transplantation, chemotherapy and infection with human immunodeficiency virus)
- Infection-associated hemophagocytosis

Vertical transmission, fetal hydrops and intrauterine fetal death (IUFD)
- Nonimmune fetal hydrops
- Congenital anemia

school-aged children with low-grade fever, malaise and the characteristic facial rash, involving the cheeks with relative circumoral pallor, that subsequently spreads to the trunk, back and extremities. Arthralgia is more frequent in adults.

Patients with underlying hematological or immunological disorders are at risk for transient aplastic crisis (TAC), due to the block of erythropoiesis with a profound reticulocytopenia. Other manifestations associated with B19 infection are illustrated in Table 113.12.

Primary maternal infection has been associated with:
- asymptomatic fetal infection;
- birth defects, particularly ocular and central nervous system abnormalities (long-term neurologic sequelae in infants with no signs at birth have been described);
- intrauterine fetal death, more common in the first and second trimester, often unassociated to hydrops;
- fetal hydrops, occurring in 1 out of 3000 births, with a mean interval of 6 weeks between the onset of maternal infection and fetal symptoms.

Maternal clinical manifestations do not influence the pregnancy outcome.

113.5.3.1 Diagnosis

Diagnosis of B19 infection is based on detecting specific IgG and IgM or viral DNA in blood or tissue samples by PCR [23].

Specific IgM are present 10–12 days after infection; IgG appear shortly afterwards and mediate lifelong immunity. Caution is needed in interpreting serology in immunodeficient individuals or in pregnant women, because they are not always able to mount an adequate antibody response.

PCR analysis of B19 DNA in serum, bone marrow (BM) and other tissues may integrate serology. In immunocompromised patients, a positive PCR test in blood indicates ongoing acute or persistent infection, in BM it may indicate either acute or previous infection. In cases of fetal complications, PCR analysis of amniotic fluid or cord blood may have a high priority for possible therapeutic interventions. Quantitative PCR allows the viral load concentration in different tissues to be quantified. However, further studies are needed to correlate the viral load with disease progression.

113.5.4 Prognosis

After intrauterine exposure to B19, the risk of fetal death is 0.1–0.3%. Information on long-term outcomes of congenital infections is limited. No studies report significant developmental delay in live-born children infected in utero, including those treated with intrauterine transfusion for B19 anemia and hydrops.

113.5.5 Therapy and Treatments

There is no specific therapy against B19 infection; some alternative interventions are however recommended, such as transfusion therapy to recover from aplastic crisis or intravenous IgG ($0.4\ g\ kg^{-1} \times 5$ days or $1\ g\ kg^{-1} \times 3$ days) to facilitate the clearance of the virus in immunocompromised patients.

If maternal B19 infection is confirmed, weekly ultrasound examinations are appropriate to monitor the fetus: if hydrops and/or signs of anemia appear, intrauterine erythrocyte transfusions can reduce the mortality rate [24].

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Fetal Infections: Congenital Syphilis, and Tuberculosis

Pier-Angelo Tovo, Carlo Scolfaro, Federica Mignone and Silvia Garazzino

114.1 Syphilis

Significant progress has been made in the diagnosis and treatment of syphilis since the identification of the causative agent, *Treponema pallidum*, in 1905. However, syphilis remains a major public health problem in many developing countries and there has been a recrudescence in developed countries during the last two decades [1].

The prevention of congenital syphilis is based on good antenatal care and routine serological screening to detect infection in pregnant women.

The newborn can be infected in utero by the transplacental passage of *T. pallidum* from an infected, untreated or inadequately treated mother, or at delivery by contact with an active genital lesion. Breastfeeding does not cause transmission. *T. pallidum* may cross the placenta from about 14 weeks of gestation. The risk of transmission increases with gestation and is inversely related to the duration of maternal infection. The vertical transmission rate in an untreated mother ranges from 70–100% during primary syphilis, 40% during early latent syphilis, and decreasing to 10% during late latent infection [2, 3].

Syphilis is classified as early and late. It is early when it is diagnosed within the first or second year after infection. Late syphilis is diagnosed one or two years after infection [3].

114.1.1 Epidemiology

The World Health Organization (WHO) estimates that 12 million people are infected with syphilis each year [4]. Untreated early syphilis during pregnancy results in perinatal death in up to 40% of cases. If acquired during the 4 years preceding pregnancy, it can cause infection of the fetus in 80% of cases.

In sub-Saharan Africa, an estimated two million women with active syphilis become pregnant each year and the infection remains mostly undetected during pregnancy. In Latin America and in the Caribbean the prevalence of syphilis in pregnant women ranges from 5 to 10%, while in Asia it is generally below 5% [2]. The rates of primary and secondary syphilis declined in the United States in the 1990s, but in the new millennium it has increased annually with 1.1/100,000 women being infected in 2007 with a peak in the 25- to 29-year-old age group (8.9/100,000). As a consequence, the rate of congenital syphilis has increased after 14 years of decline. Recently, there has also been a resurgence of syphilis in Europe [3, 4].

114.1.2 Etiology and Pathogenesis

T. pallidum is a member of the order Spirochaetales, family Spirochaetaceae and genus *Treponema*. If untreated, the infection persists despite evidence of an immune response. *T. pallidum* interacts with vascular endothelium. Its membrane 47 kDa lipoprotein activates vascular endothelial cells to upregulate the expression of intercellular adhesion molecule-1 (ICAM-1) and stimulate procoagulant activity, resulting in fibrin deposition and perivasculitis. Delayed-type hypersensivity to treponemal antigens appears later and may be related to the onset of latency [5].

114.1.3 Clinical Manifestations

Symptoms and signs of congenital syphilis within the first 2 years of age are considered as early congenital syphilis, and later clinical manifestations as late congenital syphilis.

In pregnant women, untreated or inadequately treated syphilis is associated with spontaneous abortion during the second and early third trimester, perinatal death, premature

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delivery, low birth weight, and non immune hydrops. Most affected infants are asymptomatic at birth, but two-thirds develop manifestations by 3–8 weeks. Persistent rhinitis (snuffles) is an early feature, with an infectious discharge that is sometimes blood-tinged. Other signs include a non-tender generalized lymphadenopathy, hepatosplenomegaly, anemia, leukopenia or leukocytosis, thrombocytopenia and conjugated hyperbilirubinemia. The most common skin lesion is a maculopapular rash or a vescicolo-bullous eruption, affecting the palms and soles and associated with desquamation. Glomerulonephritis followed by nephrotic syndrome with generalised edema may develop between 2 and 3 months of age. Bone lesions are not unusual, with involvement of the metaphyses and diaphyses of long bones; radiographic abnormalities can be detected in about 20% of infants with asymptomatic infection. Osteochondritis or Parrot's pseudo-paralysis is a most common and early manifestation characterised by an asymmetric, painful, flaccid paralysis of the limbs and knees.

Late congenital syphilis may involve many organs, most frequently the bones, teeth and nervous system. The stigmata of late infection include Hutchinson's triad (notched upper incisors, interstitial keratitis and eight nerve deafness), saddle nose, palatal erosions, frontal bossing, sabre tibia, rhagades, nodules and gummata.

114.1.4 Diagnosis

Syphilis can be diagnosed by direct and indirect tests (Table 114.1). Direct methods can be used for symptomatic patients with primary or secondary syphilis and include:

- the detection of *T. pallidum* by direct examination of fluid or exudation from lesions immediately after sample collection through dark-field microscopy. The sensitivity of the method (74–86%) is limited by the fact that 10^5 organism/mL are necessary for visualization. However, this method cannot be used for oral lesions because non-pathogenic oral spirochaetes can be confused with *T. pallidum* and systemic antibiotics may result in a false-negative test. Specificity is 77–100% and depends on the experience of the observer;
- direct fluorescent antibody *T. pallidum* test (DFA-TP) in smear or tissue section. This does not require motile organisms and is more sensitive and specific than dark-field microscopy, though more expensive;
- polymerase chain reaction (PCR) for the identification of treponemal DNA has been developed but not yet standardized. Can detect as few as 10 organisms in lesions or tissues [5, 6].

Indirect diagnosis is based on serological tests to detect antibodies. These tests include non-treponemal tests for screening and treponemal ones for confirmation.

- Non-treponemal tests (e.g., Venereal Disease Research Laboratory [VDRL] and Rapid Plasma Reagin [RPR]) measure both IgG and IgM antiphospholipid antibodies against lipoidal material released by damaged host cells and the treponemal cell surface. These tests become positive 6 weeks after exposure and 1–4 weeks after the appearance of the primary lesion. They usually correlate with disease activity. Results are reported quantitatively and a four-fold titer change is considered significant. Sequential serology should be performed using the same test, preferably by the same laboratory. Non-treponemal tests usually become non-reactive over time after treatment, though they may persist at low titer (serofast reaction, which occurs when a nontreponemal test reaction is overwhelmed by antigen-antibody excess) in some patients [7]. False-positive results may occur because of cross-reactivity during acute viral infections, auto-immune conditions or pregnancy. False-negative results may be due to prozone reactions.
- Treponemal tests (e.g., fluorescent treponemal antibody adsorbed [FTA-ABS], *T. pallidum* particle agglutination [TP-PA]) do not correlate with disease activity and can remain positive regardless of treatment.

Treponemal enzyme immunoassay [EIA] can be useful because it identifies IgG and IgM against *T. pallidum* [2, 3, 5, 6].

Effective prevention and detection of congenital syphilis depends on identifying the infection by routine serological screening at the first antenatal visit and at delivery. Serology should be repeated at the beginning of the third trimester in populations with a high risk of congenital syphilis (e.g., multiple sexual partners, presence of a sexually transmitted disease, drug addiction, lack of antenatal care) or in areas with a high prevalence of syphilis (Table 114.2). Information about the treatment of the sex partner should be obtained to assess the risk of re-infection. Pregnant women with syphilis should also be tested for HIV infection. No infant should be discharged from the hospital without determining the maternal serological status for syphilis.

Every infant born to a mother with reactive non-treponemal and treponemal test results should be carefully examined for signs and symptoms of congenital syphilis. A quantitative non-treponemal serological test and EIA IgM should be done on infant serum, using the same test as that for the mother to compare titre results. If the EIA IgM test is negative and other tests have titers less than four-fold higher than the mother's,

Table 114.1 Diagnostic tests for syphilis

Direct tests
• Direct examination through dark-field microscopy
• Direct fluorescent antibody *T. pallidum* test (DFA-TP)
• Polymerase chain reaction (PCR) assay
Indirect tests
• Non-treponemal tests
– Venereal Disease Research Laboratory (VDRL)
– Rapid Plasma Reagin (RPR)
• Treponemal tests
– Fluorescent treponemal antibody adsorbed (FTA-ABS)
– *T. pallidum* particle agglutination (TP-PA)
– Treponemal enzyme immunoassay (EIA)

Table 114.2 High risk of congenital syphilis

- Symptomatic newborn
- Untreated, inadequately treated or non-documented treated maternal syphilis
- Non-penicillin treatment of syphilis during pregnancy
- Treatment of maternal syphilis for less than 4 weeks before delivery
- Absence of at least a four-fold decrease in non-treponemal maternal titres after therapy
- Four-fold increase of maternal titres or infant titres at least four-fold greater than maternal titres

an asymptomatic infant should have repeat serology at 3, 6 and 15 months of age or until seroreversion.

Congenital syphilis is diagnosed if the EIA IgM test is positive and/or the non-treponemal or treponemal titres are four-fold higher than in the mother. Further investigations are mandatory: cerebrospinal fluid (CSF) examination (protein, cell count and VDRL test), hematology and liver function tests, skeletal X-rays and ophthalmological assessment. A definitive diagnosis of congenital syphilis is made if PCR and/or dark field microscopy detects *T. pallidum* in a sample from a lesion, the placenta or body fluids [5, 6–9].

114.1.5 Treatment

Several guidelines define the criteria for the correct management of infants born to mothers with syphilis. Treatments for infants with suspected congenital syphilis are listed in Table 114.3. In infants at low risk of congenital syphilis, some specialists recommend a single intramuscular dose of benzyl penicillin G (50,000 U/kg) with follow-up serology.

114.1.6 Follow-up

Treated infants should be followed-up at 3, 6 and 12 months of age until non-treponemal serology becomes non-reactive or the titer has decreased four-fold. In adequately treated children, non-treponemal titres usually decrease by 3 months and become negative by 6 months. If they persist or increase at 6–12 months of age, the infant should be investigated and treated with a further 10 day course of parenteral penicillin G.

If the mother was adequately treated, no further investigations are required for untreated asymptomatic infants. If the newborn's or the mother's status is unclear, the non-treponemal test should be repeated at 3 and 6 months and a treponemal test should be performed at 15 months [4, 7–9].

114.2 Tuberculosis

Tuberculosis (TB) is the leading cause of death from a single infectious agent and a worldwide primary health problem. In the last decades, TB has remained a major disease in developing countries, and has also become an emerging infection in industrialized areas because of sustained migration and the spread of multidrug-resistant strains. The resurgence of TB has raised particular concerns for pregnant women and their children with a dramatic increase in the incidence of TB among women of child-bearing age [10].

114.2.1 Etiology and Pathogenesis

TB infection in infants is most commonly acquired postnatally through exposure to family members with bacillary pulmonary disease. Congenital TB is rare and is caused by maternal transmission of *Mycobacterium tuberculosis* to the fetus. Fetal infection can occur by hematogenous spread of mycobacteria through the placenta, by in utero aspiration or ingestion of infected amniotic fluid, or by direct contact with maternal genital lesions at delivery. Placental involvement may result from the dissemination phase of either a primary maternal infection or reactivation of a previous infection. Disseminated disease very often complicates acute infection rather than being due to reactivation. Primary infection during pregnancy therefore implies a higher risk of congenital transmission. The latter is unusual if maternal antituberculous treatment is adequate. HIV-infected women are at greater risk of congenitally transmitting mycobacteria, as they are more frequently affected by miliary or extrapulmonary forms of TB [11].

In hematogenous congenital TB, mycobacteria reach the fetus through the umbilical vein, causing a primary lesion within the liver and the periportal lymph nodes followed by spread into the systemic circulation and the lungs. The bacilli

Table 114.3 Recommended regimens for infants with suspected congenital syphilis

Drug	Route	Dosage	Total duration	Notes
Benzyl penicillin	IV	*Infants < 4 weeks*: 50,000 U/kg/dose every 12 hours for the first 7 days of life and every 8 hours thereafter *Infants > 4 weeks*: 200,000–300,000 U/kg/day, administered as 50,000 U/kg every 4–6 hours	10–14 days	If more than one day of therapy is missed, the entire course should be restarted
Procaine penicillin G	IM	50,000 U/kg/dose once daily	10–14 days	

generally remain dormant until the pulmonary circulation and oxygenation increases after birth, when they can cause active pulmonary disease and further systemic dissemination. When congenital TB is caused by aspiration or ingestion of infected amniotic fluid, primary complexes develop in the gastrointestinal and/or respiratory tract.

114.2.2 Clinical Patterns

Symptoms and signs of congenital TB are non-specific, often mimicking common neonatal diseases, such as sepsis or other congenital or perinatal infections. Clinical manifestations may be evident at birth, but usually appear within 2–3 weeks (see Table 114.4). Typically, infected infants are born prematurely. Virtually any organ system may be involved, although the lungs, liver and gut are the most common primary infection sites. A chest X-ray is usually abnormal and there is often a miliary pattern. TB in the newborn often results in disseminated and fatal disease [12].

114.2.3 Diagnosis

The diagnosis of congenital TB is often difficult. It should be suspected in a newborn with negative tests for other congenital infections, and whose mother has risk factors for, or has been diagnosed with, TB.

The tuberculin skin reaction is unreliable in neonates, since it may be initially negative, becoming positive after a few months. However, neonates often yield positive acid-fast bacilli smears and cultures (up to 75%), as disseminated and rapidly progressive diseases lead to high bacillary loads.

Diagnosis relies on the demonstration of *M. tuberculosis* in tissues or fluids. An early morning gastric aspirate is more easily collected than sputum from infants. In the case of pulmonary TB, mycobacteria may also be demonstrated in the tracheal aspirate because of high bacillary loads in the lungs. Three consecutive samples should be obtained, but a single positive pulmonary smear should be considered indicative of TB. Data on the accuracy of polymerase chain reaction (PCR) techniques in neonates with congenital TB are limited [11, 13]. The development of interferon gamma assays has offered new possibilities for the diagnosis of latent TB infection and active disease in adults, but little is known about their performance in children, especially in those exposed perinatally. Some concerns have been expressed about the reliability of these assays in newborns and infants, given their depressed IFN-γ production.

Due to high co-infection rates, all newborns and mothers diagnosed with TB should also be tested for HIV infection.

The diagnostic criteria for distinguishing congenital TB from TB acquired postnatally requires the documentation of tuberculous lesions, plus at least one of the following: (1) onset of lesions during the first week, (2) a primary hepatic complex or caseating granulomas, (3) infection of the placenta or maternal genital tract, (4) exclusion of TB acquired after birth from the mother or other sources.

114.2.4 Therapy and Treatments

Because of its high mortality rate, the early treatment of congenital TB is mandatory. The optimal treatment regimen and its duration have yet to be established. High mycobacterial loads support using a combination of drugs (Table 114.5). One regimen starts with a combination of four antituberculosis drugs (isoniazid, rifampin, pyrazinamide and streptomycin or ethambutol) for 2 months, followed by isoniazid (INH) and rifampin (RFP) for 4 to 6-months. For sensitive strains, an alternative 9-month-regimen using only INH and RFP has been suggested. Extrapulmonary disease requires longer treatment for 12 months and some experts recommend a total treatment duration of 9–12 months, regardless of the site of infection because of the impaired immunologic defences of neonates and infants.

When there are drug-resistant strains and/or intolerance to first-line treatment, second-line drugs include fluoroquinolones (i.e., moxifloxacin, levofloxacin and ciprofloxacin), ethionamide, PAS, cycloserin, aminoglycosides (amykacin, capreomycin, kanamycin) and linezolid.

During treatment, liver function tests and serum uric acid levels should be monitored and hearing tests done when streptomycin and aminoglycosides are used. Vitamin B6 is recommended to prevent INH-induced peripheral neuropathy.

Table 114.4 Signs and symptoms of congenital tuberculosis

Common	Rare
– Hepatosplenomegaly	– Vomiting
– Respiratory distress	– Jaundice
– Fever	– Cyanosis
– Lymphadenopathy	– Apnea
– Abdominal distension	– Seizures
– Poor feeding/low weight gain	– Petechiae
– Lethargy/irritability	
– Ear discharge	
– Papular skin lesions	

Table 114.5 First-line antituberculosis drugs

Drug (generic name)	Daily dose (mg/kg)	Maximal daily dosage	Route
Isoniazid	10–15	300 mg	Oral
Rifampin	10–20	600 mg	Oral
Pyrazinamide	20–40	2 g	Oral
Streptomycin	20–40	1 g	Intramuscular
Ethambutol	15–25	2.5 g	Oral

Ethambutol administered in young infants at > 15mg/kg/day can cause optic neuritis and red-green color blindness.

A gastric aspirate should be repeated after 2 months of therapy to establish the response to specific treatment. Chest X-rays should be obtained 1–2 months after the start of therapy to estimate the extent of the disease [11, 12, 14].

114.2.5 Prevention

Key to the prevention of congenital TB is the prevention and treatment of active infection in pregnant women [12]. The treatment of active disease during pregnancy includes the standard regimen of four active drugs for the first 2 months followed by a consolidation regimen with INH and RFP for a further 4 months. No teratogenic effects have been demonstrated with first line antituberculosis drugs. Careful monitoring for clinical features of hepatitis and other side effects is recommended. Recent guidelines advocate treating latent infection during pregnancy at any gestational age with INH for 9 months, aiming at preventing the development of active disease and spread to other people.

The Bacille Calmette-Guérin vaccine is ineffective in preventing congenital tuberculosis. New vaccine development may become a key future strategy for the prevention of TB.

114.2.6 Prognosis

The early diagnosis and the institution of appropriate therapy have markedly decreased the mortality of congenital TB.

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Toxoplasmosis in the Fetus and Newborn

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115.1 Introduction

In the developed world, human toxoplasmosis is considered a benign self-limiting disease mostly detectable by specific antibody *Toxoplasma gondii*-immunoglobulin (*Tg*-Ig) testing. Patent and sometimes devastating disease may be appreciated in immune-compromised hosts and congenitally infected infants [1]. Congenital toxoplasmosis (CT) occurs almost exclusively when primary maternal infection occurs during gestation. As more than half of mothers giving birth to infected offspring could not recall an infection-related illness and no test reliably marks off infection time, there is pressure for screening. Alternative strategies include prenatal surveillance in *Tg*-Ig negative pregnant women and prophylaxis of fetal infection/damage in seroconverters, and newborn *Tg*-IgM testing on filter paper blood spots and sequelae prophylaxis in the *Tg*-IgM positive infant. Monthly or quarterly re-testing of unprotected pregnant women has been widely practised in the EU since the '70s, whereas newborn testing has been included in the New England Neonatal Screening Program (NENSP) since 1986, and feasibility confirmed across the globe.

New assays and combined multi-test strategies have dramatically improved prenatal diagnosis, while treatment relies on few out-dated poorly understood pharmaco-kinetics/dynamics drugs which showed unable to eradicate tissue cysts.

Weak proof of treatment benefits, concerns about infrastructure costs and adverse effects, differences in test and medicine local availability on markets and interpretation of study results cause wide variability in practice. Equipoise considerations are blocking randomized-placebo controlled trial realization in screening countries [2].

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Toxoplasmosis in adult is emerging as a potential serious food/water-borne disease requiring hospitalization [3], and as a major cause of retinal infection, with overall 24% of affected eyes becoming legally blind [4]. News inside host and parasite might open new opportunity of health intervention. For example the association of positive *Tg*-Ig with changes in reaction time and personality profile, the inhibitory effect on parasite replication of some antipsychotics and mood stabilizers [5, 6], parasite and host genetics, such as non-archetypal highly virulent strains in severe acute course [7], kinase polymorphism virulence linkage [8], and association of ocular disease with parental polymorphisms in COL2A1 on EU and USA [9].

115.2 Epidemiology

Nearly one-third of humans shows serological markers of acquired toxoplasmosis with variation in local prevalence according to sanitation, dietetic habits, and latitude. In the Northern hemisphere, gestational toxoplasmosis and CT prevalence range from 0.5 to 8 per 1000 susceptible pregnancies and 1 to 10 per 10,000 live newborns, respectively [1]. Unexpectedly, a ten times higher than expected CT incidence with a burden of 2300 disability-adjusted life years (DALYs) has been recently calculated in the Netherlands [10]. Incidence updating is needed in the EU as an increasing number of women is facing gestation while unprotected. In fact, depending on improved conditions on farming and food storage, and on prenatal standards of care, a progressive decrease in age-specific *Tg*-IgG prevalence has been found.

115.3 Etiology and Pathogenesis

Humans usually become infected by ingesting the tissue cyst stage of *Toxoplasma gondii* (*Tg*) in meat or the oocyst stage

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released by acutely-infected felidae in water and ground. A seronegative recipient could become infected by organ transplantation from a *Tg*-Ig positive donor. If primary infection occurs during gestation the fetus might be transplacentally contaminated. Gestational toxoplasmosis is mostly under-diagnosed. In fact, only on a few cases lymphadenopathy and/or flu-like illness, without remarkable prenatal ultrasounds (US) findings, were complained. A severe TORCH syndrome should be investigated if fetal intracranial calcification, ventricular dilatation, hepatomegaly, ascites, and/or placental thickening are detected.

In a minority of women, ocular toxoplasmosis might occur in the acute and latent phase.

With rare exceptions, highlighting the inability of the host immune response to eradicate *Tg* tissue cysts, reactivation of latent toxoplasmosis poses no threat [11, 12]. The potential for overt disease and fetal transmission during the chronic phase has been reported in severely immune-compromised pregnant women (CD4 count < 200 cells/mm^3) and on a few anedoctic cases.

The major determinant for fetal infection is the gestational age week (AW) of maternal infection [13]. Acute disease

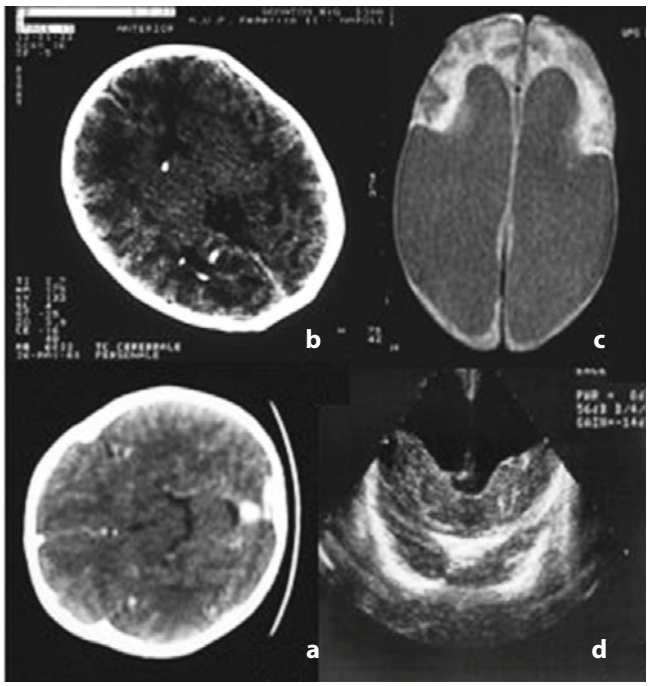

Fig. 115.1 Intracranial lesions in congenital toxoplasmosis. (**a**) Cranial CT scan showing multiple nodular calcifications near ventricular borders in a 2 month old boy with moderate onset congenital toxoplasmosis, including microcalcifications and peripheral retinochorioiditis scars on the left eye. (**b**) Cranial CT scan showing multiple sparse microcalcifications in a 43 day-old girl with asymptomatic congenital toxoplasmosis. Both children were treated for 1-year. (**c**) Massive tetra-ventricular dilation as a consequence of obstructive hydrocephalus and intracerebral calcifications in a 1 month-old infant with severe onset congenital toxoplasmosis, including bilateral chorioretinitis, and microftalmia and cataract on the right eye (CT scan on **c**, and ultrasonography on **d**). P-S regimen was applied on the mother starting from 22 AW until delivery because of positive *Tg*-PCR on amniotic fluid from a II trimester proven gestational contamination. Despite shunt placement and 1-year treatment, the child remained developmentally and neurologically severely compromised, and bilaterally blind. (Courtesy of Prof. A. Brunetti and Dr. A. D'Amico)

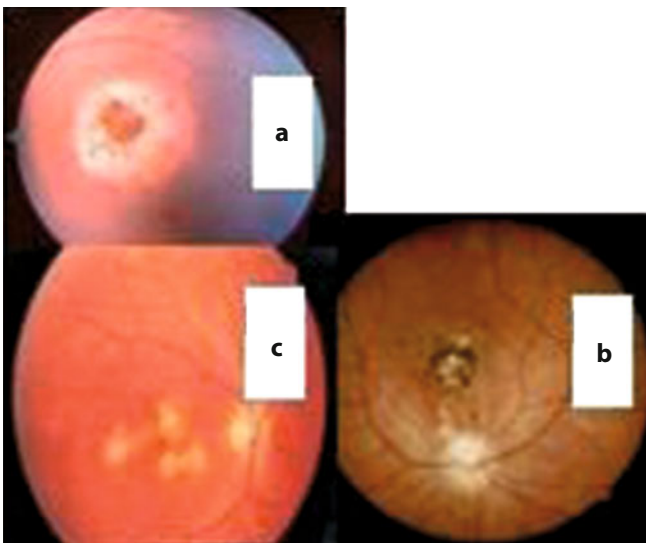

Fig. 115.2 Fundoscopic images of retinochorioiditis on patient with congenital toxoplasmosis. (**a**) Large macular lesion with a central hyperpigmented area and a semi-quiescent border on which pigmentation started developing in a 3-year old boy with presumed severe onset congenital toxoplasmosis (multiple cranial calcifications and macular retinochorioiditis were detected when he was 14 months old and referred because of "lazy eye"). (**b**) Large old chorioretinal scar with sharply demarcated and pigmented edge, and many satellite lesions on different resolution phases in a 13-year old boy with untreated severe onset congenital toxoplasmosis (misdiagnosis of sepsis at birth on a child with systemic signs, cranial calcifications and slight ventricular enlargement). Adjacent to the scar, two lesions; on the lower-temporal lesion pigmentation with sharply demarcated edges accompanied the absence of inflammation, while on the upper nasal lesion active inflammation is revealed by a yellow blurred edge. Two new inflammatory lesions on the mid- inferior. (**c**) Many small yellow-white soft blurred edges displayed acute peripheral lesions in a 11-year old girl with recently treated congenital toxoplasmosis. She was born prematurely and treated with O_2 therapy soon after birth. The diagnosis of congenital toxoplasmosis was suspected when she was 9 months old, based on the detection on the other eye of a peri-papillary scar during ophthalmologic follow-up. (Courtesy of Prof. A. Magli)

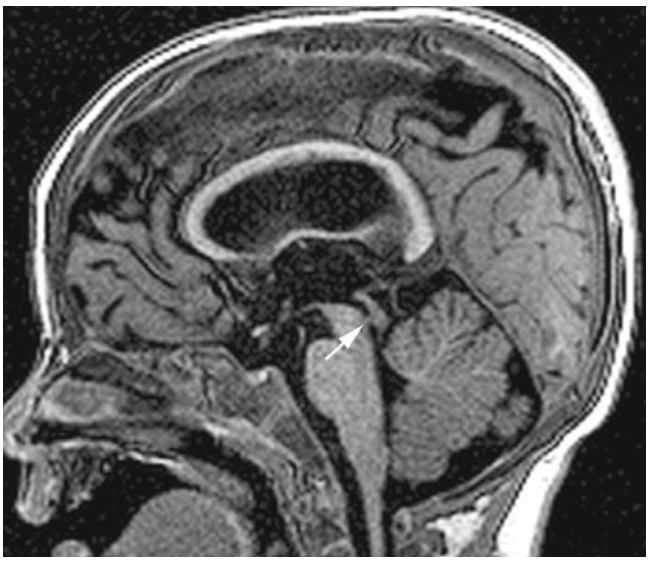

Fig. 115.3 Midline sagittal T1-3D (MPRAGE) scan (thickness = 1 mm) showing obstructed aqueduct (*white arrow*), with dilated supratentorial ventricles and normal IV ventricle. (Courtesy of Prof. A. Brunetti and Dr. A. D'Amico)

acquired ≥ 6 months before gestation shows a transmission risk approaching zero. Overall transmission rate is about 30%, with 15% at 13 AW, 44% at 26 AW, and 71% at 36 AW (Fig. 115.1). The odds of transmission increase by 12% per AW after seroconversion time. The odds of intracranial lesions markedly decrease with higher AW at maternal infection whereas the risk of ocular lesions declines less significantly (Fig. 115.2). Given the relationship, the risk of giving birth to a child with clinical signs is greatest (10%) for women acquiring toxoplasmosis between 24 and 30 AW.

In the fetus, tachyzoites show the ability to cross non-permissive biological barriers transported in antigen-presenting cells. Brain microcalcifications and ventricular dilation on neuroimaging (Figs. 115.1 and 115.3) and retinochorioiditis foci or scars on fundoscopic examination (Fig. 115.2) or on optical coherence tomography (OCT) reveal tissue damage generated by local Th1-mediated immune responses. Periaqueductal vasculitis with necrosis gradually sloughing into the ventricles might cause obstructive idrocephalus (Fig. 115.3). Long-term ocular damage is the consequence of bradyzoite to tachyzoite reconversion with at times invasion of adjacent cells (recrudescence) and secondary autoimmunity for retinal soluble antigen.

115.4 Clinical Aspects

Brain and eye damage are the hallmarks of CT, including retinochoroiditis, intracranial calcifications, and hydrocephalus. A wide clinical spectrum, including microcephaly, hypotonia, epilepsy, intracranial calcifications, electroencephalographic and/or cerebrospinal fluid abnormalities, retinochoroiditis, strasbismus, nystagmus, blindness, microftalmia, cataracts, glaucoma, optic atrophy, prematurity, intrauterine growth restriction (IUGR), hepato-splenomegaly, anemia, thrombocytopenia, fever, and lymphoadenopathy was featured in untreated cohorts [13]. Milder disease is more prevalent in the EU where overt signs were reported in a minority of congenitally infected infants enrolled in EU centers performing prenatal or neonatal screening, and attributed to differences on *Tg* genetics [14]. Of 691 infected infants, 105 (19%) presented with at least one type of clinical manifestation, 79 (14%) had ocular lesions and 49 (9%) had intracranial lesions, and only one infant died at 7 days with disseminated disease.

The diagnosis of toxoplasmosis mostly relies on serological tests. A negative post-delivery and a positive pre-conceptional *Tg*-Ig test reasonably rule out offspring risk. Conversion from a negative postconceptional test to a *Tg*-Ig M/G positive result forms a solid basis for proven gestational toxoplasmosis, and marks off maternal infection time when the between test time interval is short. Clinical usefulness of delayed treatment in order to detect high or markedly increasing *Tg*-IgG titer is reduced by the short "therapeutic window", as a recent meta-analysis reported that ≥ 3 weeks of delay in starting maternal treatment increases the odds of transmission.

On *Tg*-IgG/M positive women at their first prenatal test (≤12 weeks AW), a sequential multi-test strategy using highly sensitive IgM assays and methods examining IgG avidity or stage specificity might reasonably exclude acute infection and fetal risk on the residual IgM carrier [15]. Treatment impact on maturation of *Tg*-IgG avidity is unpredictable.

Appropriate definition of infant infection status might instead be problematic, as most of cases are normal on routine clinical examination, cranial findings are not pathognomonic and might go undetected, and serological test sensitivity is poor.

High *Tg*-IgG titer at birth might reflect transplacental transportation. Beyond the age of 12 months, *Tg*-IgG persistence or disappearance definitely confirms or rules out CT, respectively. Sequential *Tg*-IgG titer compared to the expected decay on transplacentally transported *Tg*-IgG (one half per month) or better to trend on total antibodies (IgGs) (then computing antibody load) could show between the ages of 4 to 6 months and before *Tg*-IgG level starts rising the infant is infected, thus treatment could be indicated.

Demonstration of positive *Tg*-IgM/A on newborn whole blood or sera samples is highly specific if maternal antibody leak can be ruled out. Overall IgM and IgA sensitivity was shown 52% and 55%, respectively [16]. According to maternal infection AW, IgM sensitivity increases by 29% in the first to 71% in the third trimester, and IgA sensitivity increases by 40% in the first to 64% in the third trimester. A multitest strategy including two tests (*Tg*-IgM and A) or three tests (*Tg*-IgM, A and Western blot) increases sensitivity up to 73% and 78%, respectively [17, 18]. The most informative results is a

Table 115.1 Guidelines for diagnosis of congenital toxoplasmosis

Criteria for diagnostic suspicion		
Toxoplasmosis on gestation	Maternal diagnosis precision	Proven Unproven Unlikely
	Gestational time of infection (AW)	< 13 13–35 ≥ 36
	PCR on amniotic fluid	Positive Negative
Clinical findings on newborn infant (isolated or in combination)	Ocular	Nystagmus or strabismus Posterior segment abnormalities (retinochoroiditis, optic nerve atrophy) external eye abnormalities (microphthalmos, cataract)
	Intracranial	Microcalcifications (spotty distribution, particularly) Ventricular dilatation Obstructive hydrocephalus
	Neurological	Seizures; EEG and/ or CSF abnormalities Microcephaly, macrocephaly Tone or motor dysfunction
	Systemic (TORCH syndrome)	Rash Hepatosplenomegaly Anemia, thrombocytopenia
Newborn screening	Toxo-IgM and/or IgA positivity	

Onset severity definition
– Careful pediatric examination
– Neurologic evaluation[a]
– Expert ophthalmologic examination[abc]
– Brain CT scan[de]
– Brain ultrasound (follow-up)[f]
– Auditory brain-stem response[g]

Infection status definition	
Indirect criteria	– Toxo-IgM[h] and IgA[i] ± comparative IgG WB – Toxo-IgG increase or antibody load – Toxo-IgG persistence beyond age 12 months
Direct criteria (not routinely)	– Parasite isolation or positive inoculation on mice or tissue culture (placenta) – Parasite DNA demonstration (PCR on amniotic or cerebrospinal fluid)

[a] Data sheets and narrative evaluations for each child at each examination.
[b] Appropriate examination includes cycloplegia, and under-sedation indirect fundoscopy to detect both central and peripheral lesions.
[c] Fundus photographs and optical coherence tomography (OCT), whenever the patient cooperation allowed.
[d] Without contrast medium enhancement.
[e] Higher inter-observer agreement than US and higher (five times) sensitivity.
[f] US is widely practiced to monitor short–term treatment effect.
[g] Only on infected infant.
[h] EIA and ISAGA-IgM.
[i] ELISA-IgA.

positive *Tg*-IgM result on second trimester maternal seroconversion (with a pre-test probability of 27% and a post- test probability of 79%), and a negative *Tg*-IgM result on third trimester maternal infection (with a pre-test probability of 59% and a post-test probability of 32%).

The diagnosis can be carried out prenatally at ≥18 AW and at least 4 weeks after the estimated infection time by performing PCR assay on amniotic fluid. Despite poor standardization, PCR has become standard of practice to channel prenatal management on France. The assay is highly specific, but a negative PCR result does not rule out fetal infection [19].

Ideally, suspected CT infection should be confirmed in an experienced reference laboratory, and management carried out in (or with the formal support of) a specialized center where a multidisciplinary expert team working in high quality standardized conditions and performing valid and reliable measures reported on standard format, and including retinal photographs and neuroimage report in a large database, might minimize potentials for differential misclassification [20, 21]. Particularly, on prenatal screening settings, parents and physicians felt there was less risk in prompt treatment than in deferring initiation of treatment in infants who are often undistinguishable from healthy uninfected babies. The more clinical onset definition is appropriate based on correct estimation of maternal infection time and interpretation of fetal diagnosis results, and post-test probabilities measures, the less the misdiagnosis rate, i.e., the number of treated among healthy infants and untreated among congenitally infected ones.

115.5 Differential Diagnosis

Other causes of TORCH syndrome, including Cytomegalovirus, Herpes simplex virus, Rubella and Syphilis, should be ruled out in symptomatic newborn infants by a combination of diagnostic tools. Systematic exclusion of congenital cytomegalovirus (CMV) coinfection by urine culture or PCR within 2 weeks of birth should channel appropriate onset severity definition/management, and outcome measures. A negative post-partal CMV-IgG test rules out congenital CMV, whereas a positive CMV-PCR of Guthrie card might allow differentiation between congenital and perinatal infection on case with testing delay. Exclusion of pseudo-TORCH syndrome might be indicated in multisystemic disease simulating TORCH syndrome upon which extensive screening for congenital infectious disease is negative [22].

115.6 Prognosis

In outdated studies, only 11% of asymptomatic untreated patients remained sequelae-free. Favorable cognitive, neurological, and auditory outcomes were shown in the group without substantial neurological disease at birth, and in over 72% of

moderate or severe neurological disease groups after 1-year (1-y) long postnatal treatment in the National Collaborative Chicago-Based Congenital Toxoplasmosis Study (NCCCTS) recruiting referrals all over USA [23]. New eye lesions remained undetectable in 91% of the children without neurological disease and in 64% of those with moderate or severe neurological disease. Reactivations are reported in as many as 34% of cases with early ocular involvement. More than 70% of patients in the untreated group developed new eye lesion after the first decade of life, whereas new central lesions were uncommon in 1-y treated children [24, 25].

In the EU-treated cohorts recruited through screening, outcomes were found milder than anticipated based on historical data, as 5% of patients showed serious neurological sequelae that would be apparent in the first two years of life [13]. In the European Multicentre Study on Congenital Toxoplasmosis (EMSCOT), comparable development and behavior were reported at the age of 4 years in treated infected children compared to uninfected controls [26]. Moreover, in 281 infected children, 18% had ≥1 retinochoroidal lesions, and 6% had recurrent retinochoroiditis during a median follow-up of 4.1 years, which is near to the ocular lesion prevalence reported in

Table 115.2 Treatment of *Tg* infection in pregnant women and congenital toxoplasmosis (CT) assessment

	Treatment	Dosage	Indication	Comment
Mother	Spiramycin	1 g (3 million U) every 8 h (for a total of 3 g or 9 million U per day)	a. pregnant women suspected of having acquired the infection < 16 AW b. Unproven maternal diagnosis (any AW) c. negative amniotic fluid PCR and negative US at follow-up	Until delivery not teratogenic
	Pyrimethamine (P), sulfadiazine, and folinic acid	(P) Loading dose: 50 mg every 12 h for 2 days; then 50 mg daily Sulfadiazine. Loading dose: 75 mg/ kg, followed by 50 mg/kg every 12 h (maximum, 4 g daily) Folinic acid: 10–20 mg daily (during and 1 week after (P) therapy completion)	a. women with proven infection acquired ≥ 17 AW b. women with suspected infection acquired ≥ 30 AW c. documented fetal infection (positive result of amniotic fluid PCR or abnormal US)	(P) is teratogenic half-life is 100 h Complete blood count twice a week (reversible neutropenia) (a) (b)
	Pyrimethamine (P) - sulfadoxine, and folinic acid	2 tablet (50 mg (P) 500 mg sulphadoxine each tablet) each 10 days Folinic acid: 50 mg weekly		Sulfadoxine half-life is 200 h. Potential for lethal hepatotoxicity (a) (b)
Infected infant	Pyrimethamine (P) - sulfadiazine, and folinic acid	(P) Loading dose: 2 mg/kg every 12 h for 2 days; then 1 mg/kg daily for 2 or 6 months; then same dose each alternate day or half dose daily Sulfadiazine: 50 mg/kg every 12 h Folinic acid: 20 mg each alternate day (during and 1 week after completion of (P) therapy)	Two months high dose regimen on subclinical onset; 6 months high dose regimen on symptomatic onset	1-y treatment; (P) half-life on infant is 60 h. CSF level are 10-20% of concomitant serum levels (a) (b) (c)
	Pyrimethamine (P) - sulfadoxine, and folinic acid	(P) 1.25 mg/kg – sulfadoxine 25 mg/kg each 10 days; folinic acid: 50 mg weekly	A pre-regimen with (P) and sulphadiazine for 2 months has been recently introduced. Loading dose: 1 mg/kg every 12 h for 2 days; then 1 mg/kg daily; Sulfadiazine: 50 mg/kg every 12 h for 2 months; then	For 10–24 months treatment duration; Sulfadoxine half-life on infant is 60 h (a) (b) (c)
	Prednisone	0.5 mg/kg each 12 h	a. CSF protein ≥1g/dL b. active chorioretinitis vision-threatening	Until inflammatory markers subsides (1–2 weeks apart)

Notes: Before starting sulphonamids, check for G6PDH deficit. (a) Sulphonamide discontinuation if hypersensitivity signs, including rash, Stevens-Johnson, and asthma, or micro-hematuria with urolithiasis occur; continue with pyrimethamine alone. (b) Urine alkalinisation and diuresis maintenance. (c) On absolute neutrophil count (N) < 1500/mm^3, double folinate dosage and repeat count after 3-4 days; on N count < 1000/mm^3 double folinate (20 mg/day) and stop Pyrimethamine until N >1000/mm^3; on N count < 500/mm^3, triple folinate (30 mg/day) and stop pyrimethamine until N > 1000/mm^3, repeat N count each 3–4 days. Seizure can occur on (P) regimen as overdosage consequence. (P) half-life is reduced while fenobarbital is administrated.

* None manufactured as pediatric formulation.

the National Enteric Surveillance Program NESP (20%) [27]. Nearly half of the children who eventually developed retinochoroiditis had already had their first lesion detected before age 4 months. The main retinochoroiditis determinants were US intracranial lesions and presence of clinical findings. The highest risk (80%) was found in cases with serious neurologic sequelae with the lower risk (12%) in subclinical cases. Only 2.7% of infected children developed bilateral visual impairment severe enough to affect eligibility for a driving license.

Sensorineural hearing defect is still a CT-associated sequela in untreated children. More severe ocular disease (frequency, size and multiplicity of retinochoroidal lesions) was shown in congenitally infected children in Brazil compared with EU, which might be a consequence of more virulent *Tg* genotypes predominating in Brazil, but rare in Europe [28].

In conclusion, CT is mostly a sight-threatening chronic eye disease in which occurrence of lesions during postnatal treatment contrasts with the regression or even disappearance of cranial lesions, and in which long-term follow-up to adolescence is necessary. Ophthalmologic surveillance should be more active in cases with cranial findings. Little is known about the consequence of visual impairment on life quality and comprehension subscale upon which impaired vision might impact. Further follow-up in recruited cohorts could allow better knowledge of long-term learning disabilities and behavioral problems in congenitally infected patients with and without intracranial lesions.

115.7 Therapy and Treatments

Despite the uncertainty of treatment benefits, prompt antibiotic prophylaxis in gestational toxoplasmosis to reduce vertical transmission and clinical severity, and in infected infants to reduce duration and severity and avoid permanent visual impairment is advocated. On unproven maternal infection, an expert and exhaustive discussion with parents could channel appropriate treatment choices based on presumed AW of infection and offer relief to parental anxiety.

A synergistic combination of folate inhibitors, pyrimethamine and sulfonamides, given with folinic acid is the standard regimen for gestational, fetal and congenital toxoplasmosis, with the following exceptions: 1) <16 AW treatment of early on gestation maternal infection to avoid teratogenic effects of pyrimethamine; 2) unlikely maternal diagnosis (any AW) to avoid side effects; 3) maternal infection <30 AW with negative fetal diagnosis and without US findings.

The potential for life-threatening arrhythmias exclude spiramycin from use in newborn infants [29].

Differences in treatment regimens among centers include type of sulfonamide, pyrimethamine dosage, and duration of postnatal treatment. One-year long regimen is the standard, whereas 3 months high dose continuous and 2 years low dose discontinuous were the shortest and longest regimens, respectively. On low dose discontinuous regimens, the potential for early and more severe sulphonamide side effects such as Lyell syndrome are counter-balanced by improved adherence to treatment. Dosage adjustment according to weekly complete blood count might be necessary on as much as 14–58% of cases because of neutropenia. Precautionally, glucose-6-phosphate dehydrogenase (G6PD) deficiency screening should be performed before sulfonamide administration.

Adjunctive steroids (1 mg/kg daily) might be introduced to shorten the course of vision-threatening retinochorioiditis such as that localized in the proximity of the optic nerve or the macula, and when cerebrospinal fluid (CSF) protein concentration is ≥ 1 g/dL suggesting encephalytis. Treatment reinstitution after a serological rebound is not indicated. If new eye lesions are detected, treatment reinstitution is contraindicated in the presence of healed lesions, whereas standard treatment should be given for 1–2 weeks after the resolution of acute phase in active lesions.

Guidelines for prenatal and postnatal treatment are summarized in Table 115.2.

Ventriculoperitoneal shunt placement might be necessary with frequent controls of adequate drainage suitability as patients usually require repeated shunting procedures. Compensatory strategies such as large print and "talking" books, camera magnification of materials, and spectacles to maintain best corrected visual acuity can help children compensate for the impact of the disease on cognitive function and quality of life.

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Neonatal Bacterial and Fungal Infections

Mauro Stronati and Alessandro Borghesi

116.1 Introduction

Neonatal infection is the invasion of tissue by an infective organism. This may be considered early-onset when occurring during the first 72 hours of life (very early-onset if starting during the first 12 hours), and late-onset when occurring after the first 72 hours. Infections represent a major cause of mortality and morbidity in the neonatal intensive care units (NICUs), where reported incidences range from 6% to 33% [1], and up to 40% in neonates born before 28 weeks' gestational age or with birth weight < 1000 g (extremely low birth weight, ELBW) [2]. Septicemia accounts for 45–55% of all infections, followed by lower respiratory tract infections (16–30%) and urinary tract infections (UTIs, 8–18%) [1, 3].

In NICUs, Gram-positive bacteria and particularly coagulase negative staphylococci (CONS) are the most frequent pathogens associated with nosocomial infections (55–75%), followed by Gram-negative bacteria (18–31%) and fungi (9–13%) [1, 4].

The National Institute of Child Health and Human Development (NICHD) Neonatal Research Network reported that 61% of sepsis of very low birth weight (VLBW) infants was caused by Gram-negative organisms (*E. coli* in 44% of cases) and 37% by Gram-positive organisms (*S. agalactiae* [GBS] in 11% of cases), while 70% of late-onset infections were caused by Gram-positive bacteria, 18% by Gram-negative bacteria and 12% by fungal organisms [5, 6]. CONS were the most frequently isolated late-onset pathogens, accounting for 68% of infections caused by Gram-positive organisms, and for 48% of all infections. Other Gram-positive organisms included *S. aureus* (8% of all infections), *Enterococcus* spp. and group B *Streptococcus* (GBS). *Escherichia coli*, *Klebsiella*, *Pseudomonas*, *Enterobacter* and *Serratia* spp. were the most frequently isolated Gram-negative bacteria. *Candida albicans*, accounting for 6% all infections, was the third most frequently isolated organism.

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116.2 Bacterial Infections

116.2.1 Etiology

116.2.1.1 Gram-positive Bacteria

Coagulase Negative *Staphylococcus* (CONS)

Colonization and infection by CONS usually occur nosocomially, particularly in VLBW infants [7] with an indwelling intravascular catheter (with higher risk for central venous catheters in place for prolonged times); an additional risk factor for CONS sepsis occurs with the use of lipid emulsions in solutions for parenteral nutrition [8, 9].

Staphylococcus aureus

Infections caused by *S. aureus* are most frequently nosocomial and may be superficial, involving skin (*impetigo bullosa*, staphylococcal scalded skin syndrome, breast abscess) and conjunctivae. Deep tissues may be involved (osteomyelitis and septic arthritis, pneumonia, meningitis, infection of ventricular cerebral shunts in neonates with post-hemorrhagic hydrocephalus and endocarditis) with or without associated septicemia [10] and may induce local pus (cerebral abscesses, empyema, pneumatoceles).

Infants with an indwelling central venous catheter or who have undergone surgical procedures are at increased risk of invasive *S. aureus* infections [11].

Enterococci

Account for a small proportion of nosocomial neonatal sepsis, but in the last two decades an increase in enterococcal

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

infections has been reported, as well as an alarming emergence of vancomycin-resistant Enterococci.

Group B *Streptococcus* (GBS)

GBS (*Streptococcus agalactiae*), the only species belonging to Group B of the Lancefield classification, is a Gram-positive diplococcus colonizing the enteric, genital and urinary tracts of 15–40% of pregnant women [12]. In the neonate streptococcal sepsis may be early-onset (onset during the first 6 days of life) or late-onset (onset at ≥ 7 days of life) [13].

Approximately 40–70% of infants born to GBS-colonized mothers acquire surface colonization at delivery, and, without intrapartum antibiotic prophylaxis, about 1% of colonized full-term infants develop early-onset streptococcal sepsis [13]. Maternal vaginal colonization by GBS usually precedes the development of early-onset sepsis. Other risk factors for early-onset streptococcal sepsis include maternal fever at delivery (> 38°C), premature rupture of membranes (PROM) if prolonged for more than 18 h before delivery, preterm birth, GBS urinary tract infection during pregnancy or at delivery [14]. Prematurity is the only identified risk factor for late-onset invasive streptococcal disease.

116.2.1.2 Gram-negative Bacteria

Gram-negative sepsis is less frequent than Gram-positive sepsis, but with higher mortality (36% in VLBW neonates) [6]. Gram-negative pathogens are mainly *Enterobacteriaceae* (*E. coli*, *Klebsiella*, *Serratia* spp.) and *Pseudomonas* (*P. aeruginosa*, *P. mallei*).

Enterobacteriaceae

Enterobacteriaceae colonize the enteric tract soon after birth. Invasion of the immature and/or injured mucosa and translocation of organisms to the bloodstream may cause late-onset sepsis, sometimes associated with necrotizing enterocolitis (NEC). Among Gram-negative bacteria, *E. coli* is the most frequently isolated organism in term and preterm neonates. In a survey of the Pediatric Prevention Network by Sohn et al, *E. coli* was responsible for 8.5% of all NICU infections [15]. Powdered infant formula milk has been implicated as a possible source of Gram-negative pathogens, e.g., a reported association between contamination of powdered milk by *Enterobacter sakazakii* and the occurrence of sepsis and NEC [16, 17].

Citrobacter

Citrobacter koseri, occasionally causing chorioamnionitis and urinary tract infections (UTIs) in pregnant women, may be vertically transmitted to the neonate causing sepsis and meningitis. Epidemic outbreaks in NICUs and nurseries have been described.

Pseudomonas

Organisms belonging to the *Pseudomonas* genus are preferentially transmitted through nosocomial routes of infection in NICUs. *P. aeruginosa* grows in wet environments such as humidified incubators [18] and ventilator circuits [19]. Patient-to-patient transmission through hands of healthcare workers is an important route of transmission [20]. *P. aeruginosa* may cause focal infections, e.g., pneumonia and severe conjunctivitis. In VLBW infants *P. aeruginosa* is highly virulent: Shah and Gallagher [21] reported a high incidence (39%) of systemic complications (bacteremia, meningitis, cerebral abscess, death) following *Pseudomonas* conjunctivitis. Sepsis by *P. aeruginosa* are associated with a high mortality rate (50-75%) [22].

116.2.1.3 Anaerobic Bacteria

Anaerobic bacteria account for less than 5% of all neonatal sepsis, with premature infants representing a substantial proportion of these cases. Sepsis caused by anaerobic bacteria is almost always associated with preterm maternal chorioamnionitis [23]; *Clostridium perfringens* and *Peptostreptococcus* spp. are responsible for the majority of cases of early-onset sepsis caused by anaerobic bacteria. Late onset sepsis caused by Gram-negative anaerobic bacteria (*Bacteroides fragilis*) has been associated with NEC or focal intestinal perforation. Particular virulence factors, such as pili, fimbriae and capsular polysaccharide, have been reported [24].

116.2.2 Diagnosis

Diagnosis of infection should be established soon after the onset of clinical signs of infection in order to start effective therapy as soon as possible. Signs are non-specific, but delaying antibiotic treatment may be lethal. Empirical antibiotics therapy (see below) should therefore be started before the results of investigations are available. The definitive diagnosis of infection is made by appropriate microbiological investigations, but hematological and biochemical investigations (Table 116.1) are also informative and will determine therapy.

116.2.2.1 Microbiological Tests

Isolation of a microorganism from a swab or from body fluid (blood, cerebrospinal fluid [CSF], urine) constitutes the gold standard for the diagnosis of infection and allows for adjustment of antibiotic therapy based on the antibiogram. However,

Table 116.1 Diagnostic tests in infants with suspected infection. Modified from [14]

Microbiological tests	Cultures (blood, cerebrospinal fluid, urines, swabs) Direct identification (*buffy coat*; Gram stain) Detection of bacterial antigens (electroimmunophoresis, agglutination, ELISA)
Laboratory tests	Total and differential blood count Neutrophils: immature/total C reactive protein Fibrinogen, platelet count Procalcitonin Serum A amyloid Citokines and soluble receptors (IL-6, IL-8, sCD-14, s-TREM)
Molecular diagnostics	Detection and amplification of bacterial DNA (gene coding for 16S RNA) or fungal DNA (gene coding for 18S RNA) by PCR

cultures may be falsely negative or positive, making the definitive diagnosis difficult,

False Negative

Blood cultures may be negative despite symptoms and signs of septicemia and even when disseminated bacterial or fungal infection is proven at autopsy. Sensitivity of blood cultures in neonates ranges from 8 to 73% in different studies [25].

Negative blood cultures in infants with sepsis may be due to the administration of antibiotics to the neonate or to the mother during labor, insufficient sample volume, or to a systemic inflammatory response due to non-infectious causes.

Diagnosis of sepsis has become more accurate after the introduction of sensitive and reliable culture tests as "BacTec" or "BacT Alert", that retain high sensitivity even with a small sample size (< 0.5 mL) [26].

False Positive

A single positive blood culture from a neonate with clinical signs of infection is considered evidence of sepsis. It has been suggested that two positive cultures may be required to confirm the diagnosis of CONS sepsis because of frequent contamination of samples [13]. Careful disinfection of the skin before taking blood and taking two blood cultures have been effective in reducing the contamination of cultures [27].

116.2.2.2 Laboratory Tests

Several laboratory tests can make the diagnosis of infection more accurate and timely, including hematologic tests (total and differential blood count) interleukin-6 (IL-6), IL-8, C-reactive protein (CRP), procalcitonin (PCT), serum A amyloid.

Total white blood cell count (abnormal if < 5000/μL or > 20,000/μL) and differential white blood cell count have highly variable degrees of sensitivity (17–90%) and specificity (31–100%) [28]. Thrombocytopenia (< 100,000/μL), white blood cell morphology, total neutrophil count, and the immature/total neutrophil ratio have a poor predictive value.

Blood IL-6 and IL-8 concentrations rise 12–24 hours before the onset of clinical symptoms and strongly predict infection. However, they are not routinely used by all laboratories because of the rapid fall in blood concentrations after the onset of sepsis and because of expense [29].

CRP has a 1000-fold increase in blood during an acute-phase response [25], and is not influenced by gestational age [30]. Serial measures of CRP seem to have the best predictive value during screening for sepsis, i.e., the sensitivity of a single CRP measurement is about 48–63%, increasing to 90% when the test is repeated 24–48 hours after onset of symptoms [13]. Normal serial measurements may be useful for excluding infection in a neonate with nonspecific symptoms and negative cultures [31] and in guiding the duration of antibiotic therapy.

Procalcitonin, a polypeptide precursor of calcitonin, is produced by hepatocytes and monocytes [25], has high sensitivity and specificity (87–100%) and increases during early and late onset sepsis. However, it is not generally available as an emergency diagnostic test, and is considered more accurate for late than for early onset sepsis [29, 32].

The diagnostic accuracy of the serum amyloid A has been recently evaluated. It is an acute-phase protein induced by IL-6 and IL-8 [29], and its diagnostic accuracy has been reported as higher than that of CRP in the diagnosis of both early and late onset sepsis [33, 34].

116.2.2.3 Molecular Diagnostics

The use of molecular techniques (polymerase chain reaction [PCR]) for the diagnosis of neonatal infections is still uncertain. The identification of bacterial nucleic acids in blood relies on PCR amplification of the gene for the 16S rRNA, which is present in bacteria but not in humans.

In a study of 548 samples from neonates with suspected sepsis that compared high sensitivity cultural tests (BacTec) with PCR amplification of the gene coding for the 16S rRNA, sensitivity, specificity, positive and negative predictive values were 96.0%, 99.4%, 88.9% and 99.8%, respectively. The time to obtain results was 9 hours and only 200 μL were required [35]. The high negative predictive value makes this test an important tool for limiting the excessive use of antibiotics in neonates with nonspecific symptoms.

116.2.3 Bacterial Sepsis

Sepsis is defined as the clinical syndrome characterized by a host systemic inflammatory response to invading pathogens.

For neonates, "microbiological sepsis" refers to the isolation of a microorganism from a blood culture of a baby with signs of infection and/or abnormal laboratory tests. "Clinical sepsis" is used when a neonate with evidence of infection has negative cultures [13].

116.2.3.1 Pathogenesis and Routes of Transmission

About 33–66% of neonates admitted to NICUs develop an infection (sepsis in 50%). Early onset sepsis (during the first 72–96 hours) is associated with maternal complications during pregnancy (preterm birth, premature rupture of membranes, chorioamnionitis). Pathogens associated with early onset sepsis are usually organisms of the maternal genito-urinary tract. Mortality rates range from 15 to 50% [14].

Late onset sepsis (after the first 72–96 hours of life) is usually caused by nosocomially transmitted or environmental organisms. Less commonly, maternal urinary tract organisms colonize the neonate causing later infection. Mortality rates range from 10–20% [14].

Neonates may become infected via several routes of transmission. An ascending amniotic infection is thought to be the main route of transmission for early onset sepsis. Maternal genital tract organisms (GBS, *E. coli*) ascend through the birth canal and infect the amniotic fluid either through intact amniotic membranes or, more commonly, after the rupture of membranes. Thus, vertically acquired organisms may be aspirated or swallowed by the fetus, penetrate immature mucosae into the bloodstream, and cause sepsis, often with pneumonia.

Late onset sepsis is generally acquired through horizontal or nosocomial routes of transmission (hands of healthcare workers or parents, water of ventilator circuits and incubators, biomedical instruments such as contaminated stethoscopes). Less commonly, a vertically acquired organism may colonize the neonate and cause infection.

Microorganisms pass into the bloodstream through loss of integrity of the skin and mucosae, and their translocation into deep tissues and bloodstream is increased by repeated trauma due to biomedical devices (endotracheal tubes, nasogastric tubes); alternatively, they may enter the circulation directly through a central venous catheter. Healthcare workers represent the main route of patient-to-patient transmission of microorganisms in the NICU.

Several risk factors predispose the neonate to infection and sepsis: maternal, extrinsic (nosocomial), and intrinsic (the individual susceptibility of the neonate to infection) [36].

Maternal risk factors include prolonged PROM (>18 hours before delivery), intra-amniotic infections, colonization of the maternal genital tract by GBS, maternal infections (UTI, listeriosis), obstetric invasive procedures (amniocentesis, intrauterine transfusions) and low socio-economic status.

Extrinsic risk factors include:

- Colonization. The likelihood of colonization of deep mucosal tissues (respiratory and gastrointestinal tracts) by hospital-acquired organisms is increased by the presence of biomedical devices (endotracheal tubes, nasogastric tubes). Invasive procedures that facilitate the translocation of colonizing organisms into the bloodstream include central venous catheters, total parenteral nutrition, exchange transfusion, chest drainage, prolonged intubation, frequent tracheal aspirations.
- Central venous catheters and total parenteral nutrition. The use of central venous catheters increases the risk of infection by 3.81–7-fold [4]. Freeman et al in the early 1990s reported that 14.9% of CONS neonatal infections were associated with the use of central venous catheters [9], and several subsequent studies have demonstrated a direct correlation between the duration of central venous catheter placement and the incidence of infection [1]. In a point prevalence survey by the Pediatric Prevention Network, neonates with a central venous catheter had a relative risk of infection of 3.8 (CI 2.3–6.3; $P < 0.001$) compared to infants without catheters, and the risk was 5.7 (CI 3.5–9.5; $P < 0.001$) for patients receiving total parenteral nutrition [15].
- Drugs. An association between systemic postnatal steroids for chronic lung disease and sepsis has been observed in several studies. Stoll et al [37] in a study on 371 VLBW neonates treated with dexamethasone at 14 days of life, showed that the incidence of infection was significantly higher in the treated than in the control group (22 *vs* 14%).

  The use of antagonists of the histamine type 2 receptor (H2-antagonists) for stress gastritis and gastroesophageal reflux increases the risk of both sepsis and NEC in preterm neonates [37, 38].

  Prolonged antibiotic therapy selects resistant bacteria and increases the risk of fungal infections [39]. Ampicillin, third generation cephalosporins and carbapenems select extended spectrum β-lactamase producing strains of Gram-negative bacteria that are resistant to several antibiotics, not only β-lactams. Similarly, vancomycin selects vancomycin-resistant enterococci and methicillin selects methicillin-resistant staphylococci [39].
- Formula milk. Breast milk is protective for sepsis in both term and preterm neonates. Hylander et al [40], in a study of 212 VLBW neonates, showed that the incidence of sepsis was significantly lower in those who were breast fed compared to those who were formula-fed (19.5% *vs* 32.6%; $P = 0.04$).
- Other risk factors include overcrowding, prolonged hospital stay, poor attention to good hygiene, low compliance to hand hygiene [41].

Intrinsic risk factors include:

- Birth weight and gestational age. The risk of sepsis increases with decreasing birth weight and gestational age at birth. In a survey conducted by the Neonatal Research Network, the incidence of sepsis was 43% in neonates with birth weight 401–750 g, 28% for 751–1000 g neonates, 15% for 1001–1250 g neonates, and 7% for

1251–1500 g neonates. Similarly, the incidence of sepsis was 46% for neonates < 25 weeks at birth, 29% for babies born at 25–28 weeks, 10% for babies at 29–32 weeks, and 2% for neonates > 32 weeks [6].

- Underlying diseases. Perinatal asphyxia, hyperbilirubinemia, galactosemia, malformations, patent ductus arteriosus, meconium aspiration syndrome and low Apgar scores increase the risk of infection [14].
- Immaturity of the immune system. Neonates have lower IgA levels and lower serum levels of complement factors. Several studies have demonstrated an immaturity in chemotaxis, phagocytosis, and cytotoxicity. Furthermore, maternal-to-fetal transplacental transfer of IgG occurs mainly during the third trimester of pregnancy and neonates with gestational age < 32 weeks have lower IgG levels and an immature humoral immune response [36]. For further details see Chapter 108.
- Gender. Although many studies have shown that male infants are more likely to develop neonatal infections, particularly by Gram-negative bacteria [42], studies by the NICHD Neonatal Research Network on early- [5] and late- onset sepsis [6] in VLBW infants failed to confirm this association.
- Bacteremia can be considered to be central venous catheter-related if a catheter has been in place for at least 24 hours or if it was removed less than 48 hours before the infection [3]

116.2.3.2 Clinical Features

The clinical features of neonatal sepsis are highly variable and depend on the causative organism, the site of the primary infection, neonatal age and gestation. The lower the gestational age, the more nonspecific the clinical features of sepsis. Thus, clinical features attributable to infection are nonspecific and may also be shown by non-infected neonates. Respiratory distress, disseminated intravascular coagulation, necrotizing enterocolitis, persistent pulmonary hypertension, diminished peripheral perfusion and septic shock, are frequently associated with sepsis [14]. Fanaroff et al [43] reported the following clinical signs of sepsis : apnea (55%), gastrointestinal symptoms (43%), dyspnea (29%), lethargy/hypotonia (23%), disturbances in white blood cell count (46%), metabolic acidosis (11%) hyperglycemia (10%). Bizzarro et al reported the following signs in neonates with late-onset sepsis: hypothermia (< 36.5°C; 41%), hyperglycemia (>140 mg/dL; 38%), apnea (38%), bradycardia (30%), hyperthermia (>38°C; 22%) and hypoglycemia (< 40 mg/dL; 7%) [44]. Organ-specific and systemic signs have been described (Table 116.2).

Table 116.2 Clinical signs of neonatal sepsis. Modified from [14]

General	Hypothermia, fever, poor feeding
Gastrointestinal tract	Anorexia, vomiting, diarrhea, abdominal distension, hepatomegaly
Respiratory tract	Cyanosis, tachypnea, dyspnea, apnea, respiratory distress
Cardiovascular	Pallor, cold skin, tachycardia, bradycardia, hypotension
Central nervous system	Irritability, lethargy, trembling, seizures, hyporeflexia, hypotonia, bulging fontanella, irregular breathing, acute crying
Blood	Petechiae, purpura, splenomegaly, pallor, jaundice, bleeding
Urinary tract	Oliguria

116.2.3.3 Prognosis

Both early and late onset sepsis increase hospital length stay [6], mortality and morbidity. Mortality increases with decreasing gestational age and depends on the causative microorganism, ranging between 26 and 42% for Gram-negative sepsis, 8.7 to 10.1% for Gram-positive sepsis, and 27 to 28% for fungi [1].

The Neonatal Research Network [6] reported that the overall mortality in VLBW neonates was 10%. Mortality in neonates with late onset sepsis was significantly higher than in neonates without late onset sepsis (18% *vs* 7%, $P < 0.001$). In that study, mortality was 11% in neonates with Gram-positive late onset sepsis, 36% with Gram-negative late onset sepsis, and 32% with fungal sepsis. *Pseudomonas* and *C. albicans* were associated with the highest mortality (44% and 74%, respectively).

The clinical course is usually less severe in Gram-positive compared with Gram-negative sepsis and fungi. In the Neonatal Research Network study [6], 76% of deaths due to Gram-negative late onset sepsis occurred during the first 7 days, and 24% after day 7. By contrast, only 25% of deaths due to CONS sepsis occurred during the first 7 days, and 75% after day 7. Seventy-three percent of deaths due to fungal sepsis occurred during the first 7 days and 27% after day 7 [6].

116.2.3.4 Therapy

Empirical antibiotic treatment should be started in every neonate (especially preterm neonates) with suspected infection. Antibiotics should be stopped after 48–72 h if laboratory and microbiological tests exclude initial symptoms being due to an infection [39, 42, 45].

A flow-chart for the management of the neonate with suspected sepsis is in Fig. 116.1. Antibiotic combinations should be used for empirical treatment. Ampicillin plus gentamicin is still the best antibiotic combination for early onset sepsis [39, 42]. Ampicillin is effective against enterococci, some Gram-negative bacteria (*E. coli*, *Proteus*, *Klebsiella*), GBS and *L. monocytogenes*. Aminoglycosides widen the antimicrobial spectrum, being effective against some ampicillin-resistant Enterobacteriaceae (some strains of *E. coli*, *Proteus*,

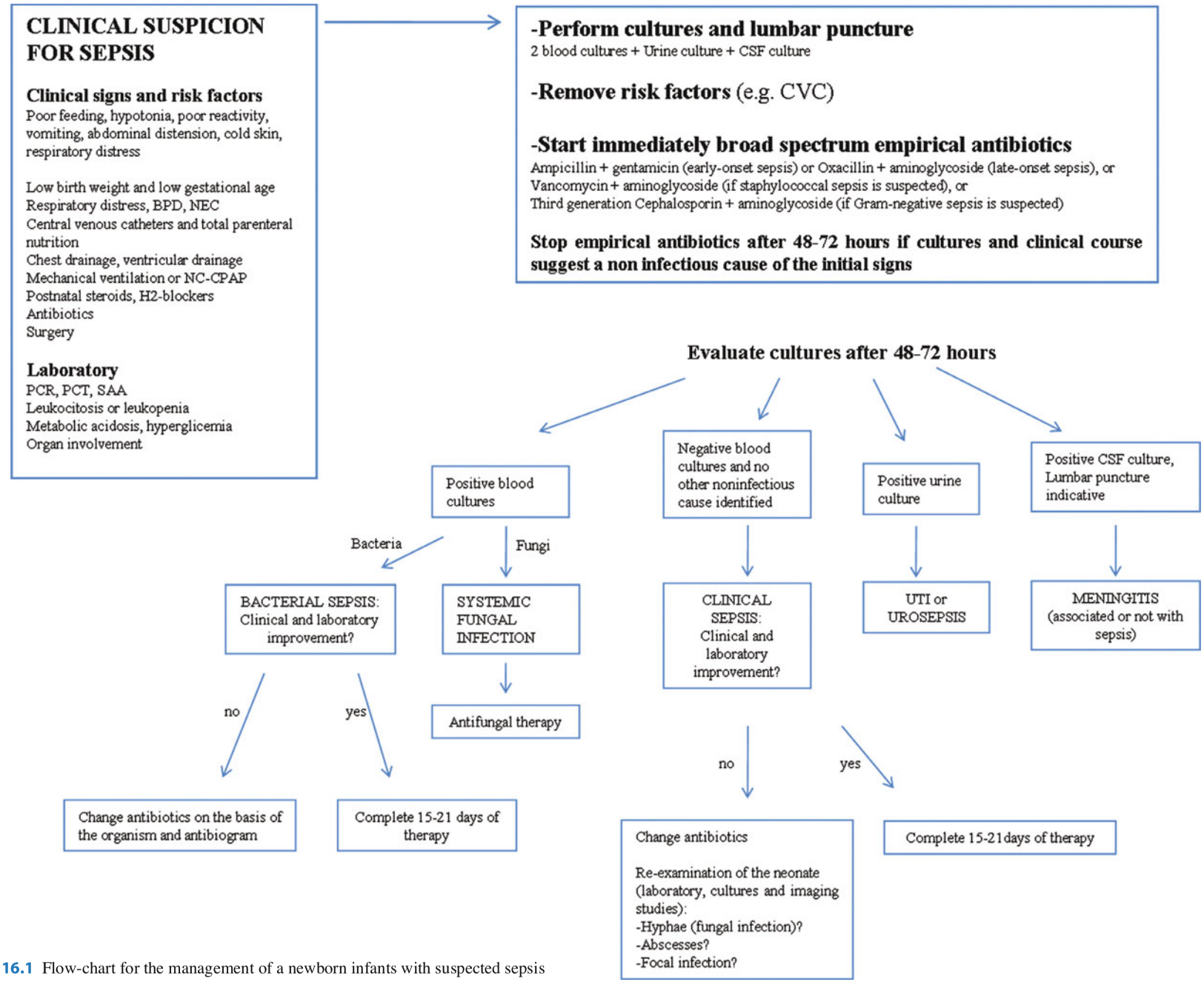


Fig. 116.1 Flow-chart for the management of a newborn infants with suspected sepsis

Klebsiella) and some ampicillin-resistant enterococci. Gentamicin is the most frequently used aminoglycoside in term and preterm neonates, and may be administered in a single daily dose. It is important to note the synergistic effect of ampicillin and gentamicin on several organisms.

Third generation cephalosporins are effective against the majority of bacterial pathogens and achieve high bactericidal concentrations in the CSF, but they should not be should not be used in the absence of proven bacterial sepsis to reduce the emergence of resistant organisms and fungal infections.

An antistaphylococcal penicillin (oxacillin or nafcillin or flucloxacillin) plus an aminoglycoside is an effective combination for late-onset sepsis [39].

When a staphylococcal infection by a methicillin-resistant *staphylococcus* is suspected or proven, vancomycin or teicoplanin should be used in combination with an aminoglycoside. Teicoplanin has fewer side effects (oto- and nephrotoxicity) and a longer half life than vancomycin, but the emergence of resistant organisms has been described less frequently with vancomycin administration.

Carbapenems may be an option for severe infections by multi-resistant organisms. Carbapenems have a very large antimicrobial spectrum (almost all Gram-negative and Gram-positive pathogens) and are resistant to known beta-lactamases. Meropenem is used because of a greater effectiveness against *Haemophilus influenzae*, Enterobacteriaceae and *Pseudomonas*. The incidence of seizures is lower than with imipenem and cilastatin.

The duration of therapy differs for Gram-positive and Gram-negative sepsis. A ten day antibiotic course seems to be reasonable for Gram-positive sepsis; longer courses may be necessary for Gram-negative sepsis and sepsis with organ involvement (2–6 weeks).

Antibiotic dosages are reported in Table 116.3.

Intravenous immunoglobulin may be useful in reducing the duration and severity of sepsis, especially in extremely low birth weight neonates. A dose of 500 mg/kg/day for 1–5 days to a maximum total dose of 2–2.5 g/kg has been suggested [46, 47].

Exchange transfusion may reduce the severity of sepsis. The volume to be transfused is 160–180 mL/kg, i.e., about twice the neonatal blood volume. Benefits depend on the removal of endotoxins, cytokines, and molecules that increase the permeability of vascular endothelium. Other advantages are attributed to the presence in the transfused blood of complement factors, antibodies and coagulation factors, and on improved lung and tissue perfusion and oxygen delivery because of the shift in the oxygen dissociation curve with the transfusion of adult hemoglobin. However, there is still little evidence of its effectiveness in reducing morbidity and mortality in patients with sepsis, and its use should be limited to patients with severe sepsis with septic shock and disseminated intravascular coagulation.

Fresh frozen plasma (10–20 mL/kg/day) may supply septic neonates with complement and coagulation factors. However there is no evidence that it benefits infants with sepsis and it is not recommended in the treatment of neonatal sepsis.

Recombinant human C activated protein modulates the clotting system and has been effectively used in adult patients with a reduction in sepsis- associated mortality. Clinical trials demonstrating safety and effectiveness of recombinant human C activated protein are lacking. Septic neonates are at high risk of bleeding, and preterm neonates are at risk of intraventricular hemorrhage. Recombinant human C activated protein increases the risk of bleeding and should be used only in the context of clinical trials [48].

Granulocyte-Colony Stimulating Factor (G-CSF) is important for differentiation, mobilization, and proliferation of bone marrow white cell precursors. A dose of 5–10 μg/kg/day subcutaneously or intravenously for 3–10 days may improve neutrophil counts in neutropenic neonates and reduce mortality in neonates with sepsis associated with severe neutropenia. Administration should be stopped for neutrophil counts greater than 20000/μL.

116.2.4 Meningitis

Isolation of a microorganism from a CSF culture is considered evidence of meningitis.

A national survey by the British Paediatric Surveillance Unit (BPSU) reported an annual incidence of viral and bacterial neonatal meningitis of 0.39/1000 births [49]. Etiology, risk factors, and pathogenesis of neonatal meningitis are similar to those for neonatal sepsis.

Early-onset meningitis (onset before the first 7 days of life) is usually caused by vaginal microorganisms transmitted vertically to the fetus (GBS, *E. coli*, *L. monocytogenes*). Late-onset meningitis (onset after the first 7 days of life) is caused by community or nosocomially acquired organisms (mainly Gram-negative bacteria and staphylococci) [13].

In a survey by the British Pediatric Surveillance Unit of 274 neonates (age < 28 days) treated for meningitis during the years 1996–1997, the most frequently isolated organism was GBS (25%), followed by *E. coli* (10%), *L. monocytogenes* (3%), other Gram-positive bacilli (6%), other Gram-negative bacilli (4%), *S. pneumoniae* (3%), and *C. albicans* (2%) [49]. There was a viral etiology in 7% of cases, and no organism was isolated in 35% of cases.

Stoll et al [50] reported on 9,641 VLBW newborn infants born at centers of the NICHD Neonatal Research Network. Gram-positive bacteria (63%) were most frequently associated with the first episode of late-onset meningitis and CONS accounted for 29% of cases. *Enterococcus* spp. accounted for 13%, *S. aureus* for 8%, and GBS for 7% of cases. Gram-negative bacteria were associated with 19% of cases of late-onset meningitis (*E. coli* accounting for 7%). Fungal organisms were associated with 18% of cases and *C. albicans* accounted for 13% of cases.

Table 116.3 Suggested dosage schedules for antibiotics used in newborns[a]

		Dosage (mg/kg) and interval of administration				
		Weight <1200 g[f] Age	Weight 1200–2000 g Age		Weight >2000 Age	
Antibiotic	Route	0–4 Week	0–7 Days	>7 Days	0–7 Days	>7 Days
Amikacin (SDD)[b]	i.v., i.m.	7.5 q12h	7.5 q12h	7.5 q8h	10 q12h	10 q8h
Amikacin (ODD)[b]	i.v., i.m.	18 q48h	16 q36h	15 q24h	15 q24h	15 q24h
Ampicillin (meningitis)	i.v., i.m.	50 q12h	50 q12h	50 q8h	50 q8h	50 q6h
Ampicillin (otherinfections)	i.v., i.m.	25 q12h	25 q12h	25 q8h	25 q8h	25 q6h
Aztreonam	i.v., i.m.	30 q12h	30 q12h	30 q8h	30 q8h	30 q6h
Cefazolin	i.v., i.m.	20 q12h	20 q12h	20 q12h	20 q12h	20 q8h
Cefepime	i.v., i.m.	50 q12h	50 q12h	50 q8h	50 q12h	50 q8h
Cefotaxime	i.v., i.m.	50 q12h	50 q12h	50 q8h	50 q12h	50 q8h
Ceftazidime	i.v., i.m.	50 q12h	50 q12h	50 q8h	50 q8h	50 q8h
Ceftriaxone	i.v., i.m.	50 q24h	50 q24h	50 q24h	50 q24h	75 q24h
Cephalothin	i.v.	20 q12h	20 q12h	20 q8h	20 q8h	20q6h
Chloramphenicol[b]	i.v., p.o.	25 q24h	25 q24h	25 q24h	25 q24h	25 q12h
Ciprofloxacin[c]	i.v.	-	-	10-20 q24h	-	20-30 q12h
Clindamycin	i.v., i.m., p.o.	5 q12h	5 q12h	5 q8h	5 q8h	5 q6h
Erythromycin	p.o.	10 q12h	10 q12h	10 q8h	10 q12h	10 q8h
Gentamicin (SDD)[b]	i.v., i.m.	2.5 q18h	2.5 q12h	2.5 q8h	2.5 q12h	2.5 q8h
Gentamicin (ODD)[b]	i.v., i.m.	5 q48h	4 q36h	4 q24h	4 q24h	4 q24h
Imipenem	i.v., i.m.	-	20 q12h	20 q12h	20 q12h	20 q8h
Linezolid	i.v.	-	10 q12h	10 q8h	10 q12h	10 q8h
Methicillin(meningitis)	i.v., i.m.	50 q12h	50 q12h	50 q8h	50 q8h	50 q6h
Methicillin (other infections)	i.v., i.m.	25 q12h	25 q12h	25 q8h	25 q8h	25 q6h
Metronidazole[d]	i.v., p.o.	7.5 q48h	7.5 q24h	7.5 q12h	7.5 q12h	15 q12h
Mezlocillin	i.v., i.m.	75 q12h	75 q12h	75 q8h	75 q12h	75 q8h
Meropenem[e]	i.v., i.m.	20 q12h	-	20 q12h	20 q12h	20 q8h
Nafcillin	i.v.	25 q12h	25 q12h	25 q8h	25 q8h	37.5 q6h
Netilmicin (SDD)[b]	i.v., i.m.	2.5 q18h	2.5 q12h	2.5 q8h	2.5 q12h	2.5 q8h
Netilmicin (ODD)	i.v., i.m.	5 q48h	4 q36h	4 q24h	4 q24h	4 q24h
Oxacillin	i.v., i.m.	25 q12h	25 q12h	25 q8h	25 q8h	37.5 q6h
PenicillinG (units)						
meningitis	i.v.	50,000 q12h	50,000 q12h	50,000 q8h	50,000 q8h	50,000 q6h
otherinfections	i.v.	25,000 q12h	25,000 q12h	25,000 q8h	25,000 q8h	25,000 q6h
Penicillinbenzathine(units)	i.m.	-	50,000 1 dose	50,000 1 dose	50,000 1 dose	50,000 1 dose
Penicillinprocaine(units)	i.m.	-	50,000 q24h	50,000 q24h	50,000 q24h	50,000 q24h
Piperacillin	i.v., i.m.	-	50-75 q12h	50-75 q8h	50-75 q8h	50-75 q6h
Piperacillin/tazobactam	i.v., i.m.	-	50-75 q12h	50-75 q8h	50-75 q8h	50-75 q6h
Rifampin	p.o., i.v.	-	10 q24h	10 q24h	10 q24h	10 q24h
Ticarcillin	i.v., i.m.	75 q12h	75 q12h	75 q8h	75 q8h	75 q6h
Ticarcillin-clavulanate	i.v., i.m.	75 q12h	75 q12h	75 q8h	75 q8h	75 q6h
Tobramicin(SDD)[b]	i.v., i.m.	2.5 q18h	2 q12h	2 q8h	2 q12h	2 q8h
Tobramicin(ODD)	i.v., i.m.	5 q48h	4 q36h	4 q24h	4 q24h	4 q24h
Vancomycinb	i.v.	15 q24h	10 q12h	10 q12h	10 q8h	10 q8h

[a] This table was published in Sáez-Llorens X, McCracken GH Jr (2006) Clinical Pharmacology of Antibacterial Agents. In: Remington JS, Klein JO, Wilson CB, Baker CJ (eds) Infectious diseases of the fetus and newborn infant, 6th edn, Elsevier Saunders, Philadelphia, pp 1223-1267.

[b] Adjustments of further dosing intervals should be based on aminoglycoside half-lives calculated after serum peak and trough concentrations measurements.

[c] Doses suggested based on anecdotal clinical experience.

[d] A loading intravenous dose of 15 mg/kg followed 24 hours later (term infants) and 48 hours later (preterm infants) by 7.5 mg/kg every 12 hours has been suggested by other investigators.

[e] Dosages of meropenem suggested are the same as those of imipenem.

[f] Data from Prober CG, Stevenson DK, Benitz WE (1990) The use of antibiotics in neonates weighing less than 1200 grams. Pediatr Infect Dis J 9:111-121.

i.m. intramuscular, *i.v.* intravenous, *ODD* once-daily dosing, *p.o.* oral, *SDD* standard daily dosing.

Frequently, meningitis is associated with, or is a complication of, neonatal sepsis, but there may be meningitis without bacteremia [51, 52]. In the study of the NICHD Neonatal Research Network, meningitis occurred in 7% VLBW neonates with positive blood cultures compared with 1.5% with negative blood cultures (P <0,001), about one third of neonates with meningitis (45/134) had negative blood cultures [50].

Microorganisms may reach the central nervous system and the meninges through breaks in the skin and consequent invasion of soft tissues and cranial sutures, but in many cases organisms reach the choroid plexus by a hematogenous route [42].

The clinical diagnosis of meningitis in the neonate is often difficult (especially in the preterm neonate) because clinical signs are often nonspecific and subtle. Meningitis can occur without systemic spread of the microorganism, and sometimes it is suspected late during the course of the disease when antibiotic treatment has already been started. Thus, the risk of underestimating the incidence of meningitis and of undertreating an infant with meningitis is very high [53].

Clinical signs of neonatal meningitis are similar to those of neonatal sepsis: fever, lethargy, vomiting, anorexia, respiratory distress, apnea, seizures, irritability, jaundice, bulging fontanelle, and neck stiffness. Perlman et al [54] reported 10 cases of late onset meningitis where the age at onset was 20 ± 14 days. Clinical signs were nonspecific: apnea and bradycardia in eight neonates, abdominal distension in 5, hyponatremia (< 130 mEq/L), oliguria and weight gain (syndrome of inappropriate secretion of antidiuretic hormone) in three. Specific signs were observed in three neonates: seizures in two and 3rd cranial nerve palsy in one. Cranial US abnormalities were found in seven out of 10 neonates (70%) and included: progressive enlargement of cerebral ventricles (six cases), thalamic echolucency (three cases), ventriculitis (four cases) and cystic leukomalacia (one case). Placement of a ventricular shunt was necessary in all six cases with enlargement of cerebral ventricles.

The diagnosis of meningitis depends on lumbar puncture for examination of the cerebrospinal fluid (cell count, chemistry and cultures). Imaging techniques (ultrasonography, magnetic resonance imaging) are mainly useful for prognosis or to establish whether there are complications.

There is debate about whether a lumbar puncture should be always performed in the investigation of an infant with suspected sepsis. Although cardiovascular and respiratory instability may often preclude the immediate performance of the procedure, the risk of undertreatment is very high. Several authors recommend examination of the cerebrospinal fluid as routine during the investigation of suspected sepsis and not only when meningitis is suspected [52].

Garges et al [51] evaluated the diagnostic accuracy of cell count and chemistry of the cerebrospinal fluid in 9,111 neonates ≥ 34 weeks: 95 had a microbiological diagnosis of meningitis. In their study, neither white blood cells nor protein or glucose levels in cerebrospinal fluid were accurate predictors of neonatal meningitis. Infants with culture proven meningitis had a mean of 477 white blood cells/mm^3 (0–15,900; interquartile range 38–1950) in the cerebrospinal fluid, higher than infants without meningitis. However, the authors observed that about 5% of infants with bacterial meningitis had 0–1 white blood cells/mm^3 in the cerebrospinal fluid, and about 10% of infants with bacterial meningitis had a white blood cell count in the cerebrospinal fluid ≤ 3/mm^3. The sensitivity and specificity for bacterial meningitis of a white blood cell count in the cerebrospinal fluid > 0/ mm^3 were 97% and 11%, respectively. If a cut-off value of > 21/mm^3 was used, sensitivity and specificity were 79% and 81% respectively. Glucose and protein levels are also very variable and poor predictors of culture-proven meningitis. However, the cell content and biochemical examination of the cerebrospinal fluid remains invaluable in the management of an infant with suspected meningitis while culture results are awaited. Suspected bacterial or fungal meningitis should always be confirmed by a positive cerebrospinal fluid culture.

The choice of antibiotics is the main tool for the treatment of meningitis, and should take account of potential pathogens (maternal or nosocomial) and their spectrum of sensitivity, based on patterns of sensitivity of previous isolates). To treat all potential pathogens, a combination of three antibiotics should be considered for initial empirical antibiotic therapy (usually ampicillin with a third generation cephalosporin and an aminoglycoside). Once culture results are available, antibiotic therapy can be modified and one antibiotic can be withdrawn on the basis of the isolated organism and its sensitivity pattern.

Ampicillin is effective against GBS and *L. monocytogenes*, which is resistant to third generation cephalosporins. In GBS meningitis, suggested by Gram stain of the cerebrospinal fluid or confirmed by culture, high doses of ampicillin (up to 300–400 mg/kg/day) or penicillin (up to 450,000 U/kg/day) should be used in combination with an aminoglycoside (usually gentamicin) [55].

Third generation cephalosporins are effective against several Gram-negative bacteria and rapidly achieve therapeutic concentrations in the cerebrospinal fluid (50–100-fold the MIC). This is in contrast to other antibiotics, such as aminoglycosides (which reach concentrations 2.5-fold the MIC) [13]. Cefotaxime is effective against the great majority of Gram-negative pathogens but not against *P. aeruginosa* (which is sensitive to ceftazidime).

For multiresistant organisms, meropenem together with an aminoglycoside may be a reasonable choice.

Antibiotic therapy should be started as soon as possible and continued for at least 21 days. The effectiveness of the initial empirical antibiotic therapy should be proven by a negative cerebrospinal fluid culture, performed 24–48 h after the onset of symptoms and a lumbar puncture for cerebrospinal fluid culture before the withdrawal of the antibiotic treatment. If the cerebrospinal fluid is not sterile at 24–48 hours, a focal infection of the brain (cerebral abscess, subdural empyema,

obstructive ventriculitis) should be suspected and excluded by imaging techniques [55].

An herpetic encephalitis should be always considered in a neonate with suspected meningitis. Aciclovir (20–30 mg/kg every 8 hours) should be started when Gram staining of the cerebrospinal fluid is negative and should be withdrawn when the herpetic infection has been excluded [55].

No consensus has yet been reached on the effectiveness of corticosteroid therapy with dexamethasone to improve the prognosis of infants with meningitis, and no randomized clinical trials have been performed.

Neonatal meningitis has a high mortality and morbidity. In the BPSU survey [49], the mortality rate for neonates with meningitis during 1996–7 was 6.6%, although the figure was high, it was less than the 19.8% reported by a previous study in 1985–1987.

The neurologic prognosis following neonatal meningitis is generally poor. In a study of 274 babies, who developed meiningitis at ≤ 28 days, there were mild-to-moderate neurologic disabilities in 12% of babies with birth weight > 2500 g, 31% for babies with birth weight 1500–2499 g and 44% for babies with birth weight < 1500 g [49]; 7.3% had severe disability, 18.2% moderate disability, and 24.1% mild disability [56]. Neurologic sequelae following neonatal meningitis include learning and language defects, motor disorders, seizures, hearing and visual defects, and behavioral disturbances.

116.2.5 Pneumonia

Neonatal pneumonia may be of early or late onset. Some authors define early-onset pneumonia as pneumonia during the first 48 hours of life, and late-onset pneumonia after the first 48 hours of life. Other reports define pneumonia as early-onset when arising during the first 7 days of life and late-onset when onset as occurring after the first week of life.

Early-onset neonatal pneumonia is caused by pathogens that colonize the birth canal or infect the amniotic fluid (especially after prolonged rupture of membranes or in the presence of chorioamnionitis) and that are inhaled by the fetus during labor. Symptoms may present at birth as neonatal respiratory distress or appear during the first days of life. Neonatal pneumonia is often associated with fetal asphyxia; the mechanism for this association is not certain but some authors hypothesize that organisms are inhaled during gasping movements caused by asphyxia [57, 58].

Pathogens may be acquired in utero and localize to the fetal lung causing congenital pneumonia. Congenital pneumonia is rarely a focal infection. It presents most often as a part of a systemic congenital infection, acquired transplacentally because of a systemic maternal infection associated with chorioamnionitis or, less frequently, in association with asymptomatic maternal infections, e.g., human immunodeficiency virus, cytomegalovirus, *L. monocytogenes*, *M. tuberculosis*, *T. pallidum* may cause congenital pneumonia) [57].

The term intrauterine pneumonia defines an inflammatory disease of the lungs, which is frequently associated with low Apgar scores, respiratory distress syndrome, or even intrauterine death of the fetus. It is usually diagnosed at autopsy in stillbirths or in babies dying during the first days of life. The etiology may be infectious (amniotic infection) or non-infectious (fetal asphyxia) [59].

Late-onset neonatal pneumonia may be acquired or may represent the clinical evolution of an infection acquired in utero.

Pathogens that cause pneumonia in the neonate are similar to those responsible for sepsis and meningitis [57, 58]. In one study of 261 neonates admitted during the first 4 days of life with respiratory distress, cultures of tracheal aspirates, blood and cerebrospinal-fluid demonstrated a prevalence of Gram-positive bacteria (71%) compared with Gram-negative bacteria (29%). GBS (19%) was the most frequently isolated Gram-positive organism, followed by *S. Epidermidis* (13%), *S. aureus* (10%), and *S. Pneumoniae* (10%). Among Gram-negative bacteria, *E. coli* was the most frequently isolated (13%), followed by *H. influenzae* (6.5%), and *Proteus mirabilis* (6.5%) [60]. The WHO young infant study (studying neonates aged 7–29 days) reported *S. pneumoniae* as the most frequent etiologic agent of late-onset community-acquired neonatal pneumonia, followed by *S. aureus*, *Klebsiella*, and *S. epidermidis* [61].

Other causes of neonatal pneumonia are:

- *Treponema pallidum*, frequently associated with a poor clinical condition and severe hypoxemia.
- *Chlamydia trachomatis*, causing a characteristic form of pneumonia with hyperinflation and diffuse bilateral infiltrates of the lungs on chest X-ray, sometimes with a peripheral blood eosinophilia and the diagnosis is confirmed by an increase in anti-*Chlamydia* IgM.
- *Mycobacterium tuberculosis*, acquired transplacentally or following inhalation of amniotic fluid, and causing pneumonia with a nonspecific, clinical course, which may be chronic or acute and which is characterized by multiorgan involvement.
- *L. monocytogenes*, responsible for fulminant sepsis, sometimes associated with pneumonia [58].

The differential diagnosis of neonatal respiratory distress should include viral pneumonia as possible etiology. Viruses causing pneumonia in the neonate include respiratory syncytial virus, metapneumovirus, influenzavirus, parainfluenzavirus and adenovirus.

Clinical manifestations of neonatal pneumonia include focal signs of infection (e.g., respiratory distress) and general signs of infection (poor feeding, lethargy, hypothermia or hyperthermia) [62]. In three studies of neonates with radiologically confirmed pneumonia, signs included tachypnea (60–89%) and increased respiratory effort (in more than 80%) [63–65].

The differential diagnosis of neonatal pneumonia includes diseases associated with respiratory distress (e.g., hyaline

membrane disease, transient tachypnea of the newborn, meconium aspiration syndrome, and asphyxia). Nonspecific signs of infection are similar to those of other neonatal infections.

Difficulties in the diagnosis of neonatal infections depend on the poor specificity of clinical signs, imaging techniques, and laboratory tests. Guidelines from the Centers for Disease Control and Prevention-National Nosocomial Infections Surveillance System included criteria for the diagnosis of pneumonia for infants aged <1 year [66]. The diagnosis of pneumonia is made on the basis of a progressive infiltrate on chest radiography associated with the isolation of a pathogen. However, pulmonary infiltrates on chest radiography may be due to other disease processes: meconium aspiration syndrome, atelectasis and bronchopulmonary dysplasia [66].

In ventilated patients, ventilator-associated pneumonia (VAP) is an extremely difficult diagnosis, particularly in preterm infants. Stolfi et al [3] defined VAP as a pneumonia in a ventilated infant in the presence of the following: a) onset after at least 24 h of mechanical ventilation; and/or b) onset not later than 48 hours after withdrawal of mechanical ventilation. Apisarnthanarak et al [67] reported 211 newborns ventilated for more than 48 h: signs of VAP included hypothermia (77%), tachypnea >75/min (65%), increase in bronchial secretions (50%), bradycardia <100/min (35%), fever (23%) and apnea (15%). Laboratory findings included purulent tracheal aspirate (> 25 leukocytes per high power field) (46%) and thrombocytopenia (15%).

Mechanical ventilation is the greatest risk factor for nosocomial pneumonia [59]. Humidification systems and ventilator circuits represent a source of organisms, mainly *P. aeruginosa*. In a study on 170 newborn infants ventilated for more than 48 h, Petdachai [68] found that an umbilical catheter (P = 0.007), respiratory distress syndrome (P = 0.03) and an orogastric tube (P = 0.01) were all independent risk factors for VAP.

Empiric treatment is based on the combination of two antibiotics, ampicillin with an aminoglycoside (usually gentamicin). If a staphylococcal infection is suspected (pneumatoceles, empyema, skin abscesses or pustules, or associated omphalitis) an antistaphylococcal penicillin (cloxacillin, flucloxacillin) or vancomycin should be used instead of ampicillin. Before antibiotic treatment, blood and urine cultures should always be performed to guide treatment in case first line empiric antibiotic therapy is ineffective. Duration of the antibiotic course depends on the microorganism: Gram-negative or GBS pneumonia should be treated for at least 10 days, but longer courses are needed for staphylococcal pneumonia (3–6 weeks). *C. trachomatis* pneumonia should be treated with a macrolide (erythromycin 50 mg/kg/day in 4 doses) for 2 weeks.

Treatment of pneumonia should include the treatment of hypoxemia (supplemental oxygen and, if necessary, mechanical ventilation), drainage of pleural purulent effusions, and the maintenance of hydration status and ion balance.

116.2.6 Urinary Tract Infections (UTI)

UTI is generally defined as the presence of an organism in the urinary tract, which is usually sterile. Urosepsis is the isolation of the same microorganism from urines and blood.

In the neonate, the incidence of UTIs is 0.1–1% increasing to 14% in febrile neonates [69]. During the first months of life, the incidence is higher in males than in females, and this ratio is reversed after the third month [70].

Kanellopoulos et al [69] studied 62 term neonates with UTI (51 males and 11 females). The pathogen most frequently isolated was *E. coli* (73%) followed by *Proteus mirabilis*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Morganella morganii*. Other potential pathogens for UTIs include GBS, *S. aureus*, *Enterococcus* spp. [71]. *E. coli* is most frequently isolated in infants without vesico-ureteral reflux, while in the neonates with vesico-ureteral reflux other Gram-negative bacteria are most frequently isolated.

During the first 48–72 h, the urine is usually sterile. In a retrospective study of 369 neonates with signs of infection by Tamim et al, blood and urine samples were cultured during the first 24 h: no urine cultures were positive, while blood culture was positive in eight neonates (2%) [72].

Lower urinary tract colonization after birth is the first step in the pathogenesis of a UTI in a neonate with risk factors for UTIs. Such risk factors include obstructive uropathies (malformations and functional abnormalities of the urinary tract, including urethral valves and phimosis, reported in up to 4% of cases) and vesico-ureteral reflux, reported in 25–40% of cases [69, 70]. A UTI is often the first clinical sign of a urinary tract malformation.

During the neonatal period, UTIs are characterized by an increased incidence of ascending infection and associated sepsis [71]; organisms that enter the lower urinary tract may easily reach the upper urinary tract and infect the kidney, leading to a systemic infection (urosepsis), or, less frequently, the kidney may represent the secondary localization of a sepsis [73].

Clinical signs of UTIs overlap with those of sepsis; therefore, every neonate with age > 3 days with suspected sepsis should undergo diagnostic evaluation for UTIs [74]. The presence of fever has been always considered in clinical practice as an indirect sign of upper urinary tract involvement [71].

Kanellopoulos et al [69] reported the most common signs of UTI during the neonatal age as fever (77% of cases), poor feeding (48%), lethargy (26%), jaundice (18%), diarrhea (13%), vomiting (8%) and failure to thrive (7%). In their study, the mean age at onset was 20 days for males and 18 days for females. 24% of neonates (14/51 males, 1/11 female) had vesico-ureteral reflux; there was pyelonephritis in 39% , and urinary tract malformations in 8% (including hydronephrosis and uretero-hydronephrosis, pyelectasis, double collecting system, posterior urethral valves).

UTI should be excluded for every neonate with nonspecific clinical signs, such as poor feeding and failure to thrive.

The diagnosis of UTI should be confirmed by positive urine culture. As urines may be easily contaminated, UTI is defined as the isolation of at least 100 colony forming units/mL (CFU/mL) from a specimen obtained by suprapubic bladder aspiration, or at least 10,000 CFU/mL from a sterile bladder catheterization [74]. In preterm infants, lower colony counts may indicate a UTI, especially in the case of Gram-negative bacteria or *Candida* spp. [13]. However, Hoberman et al [75] found that 65% of cultures with bacterial counts of 10,000–49,000 CFU/mL yielded Gram-positive bacteria or mixed flora, both suggestive of contamination rather than infection. Specificity of microbiological diagnosis may be improved by indirect signs of UTI (pyuria, bacteriuria).The false positive rate is further increased by using bags for urine specimens [71]. Urinary leukocyte esterase, nitrites, and leukocyte count and an increase in blood inflammatory markers suggest upper UTI. Sepsis and meningitis are frequently associated with UTI in the neonate, and a blood culture should be always performed before starting antibiotic. A lumbar puncture should be considered for febrile babies [73].

Treatment should be started immediately after taking blood for culture. Sepsis cannot be excluded until blood cultures results are available and the initial empirical antibiotic therapy for UTIs is identical to that for sepsis [73]. A penicillin (ampicillin) and an aminoglycoside are the first choice antibiotics; culture results and antibiograms should guide subsequent changes of treatment. Therapy should last for 10–14 days, but longer courses may be needed for relapses or malformations. A second urine culture should be taken 48 h after starting therapy to assess the effectiveness of treatment [73].

About 25–40% of infants with UTI suffer from vesico-ureteric reflux, defined as retrograde flow of urine in the ureters and/or the collecting system of the kidney. This is associated with a high risk of pyelonephritis, which, especially when relapsing, is associated with renal scarring, hypertension, and chronic renal insufficiency [76, 77]. The American Academy of Pediatrics recommended a renal ultrasound (US) and voiding cystourethrogram in all infants with a febrile UTI during the first 24 months of life [76].

A renal US should be performed in all neonates with UTI. Although its ability to detect scars in the renal parenchyma is limited, it allows for detection of dilation of the renal collecting system and the exclusion of renal abscesses or obstructive diseases [78].

A voiding cystourethrogram will detect vesico-ureteral reflux and assessment of its severity (grades I to V, from mildest to severe). It should be performed within the first 3–6 weeks in all neonates with a febrile UTI. As the risk for relapse is high in the presence of a vesico-ureteral reflux, antibiotic prophylaxis should be started after antibiotic treatment until completion of imaging studies to assess reflux [76, 79]. Prophylaxis should be continued in neonates with vesico-ureteral reflux. Prophylaxis should be performed with a single daily oral antibiotic dose given at night at half of the normal dosage. Cephalosporins and amoxicillin are the most used antibiotics.

Antibiotic prophylaxis may be appropriate for low grade reflux (grades I-II) which frequently resolves spontaneously during the first year of life, but grade V reflux that should be treated surgically. Treatment for grade III, IV or V reflux should be decided with the involvement of a pediatric urologist and should take account of the probability of spontaneous resolution, the presence of renal scars, and the failure of medical treatment (poor compliance, appearing of resistance to antibiotics, side effects of antibiotics).

Renal scintigraphy (with dimercaptosuccinic acid, DMSA) is indicated in all infants with grade III, IV or V reflux or in the presence of renal injury at US.

The follow-up of a UTI includes urine culture and urinalysis for every unexplained febrile episode. The presence of vesico-ureteral reflux should be monitored (annually or less frequently) until resolution or surgical correction.

116.2.7 Osteomyelitis and Septic Arthritis

Osteomyelitis is the infection of the bone and/or the bone marrow. It may be acute or chronic, but in the neonates the course is usually acute. Septic arthritis is the infection of a joint and may present in isolation or as a complication of osteomyelitis [80]. Osteomyelitis is infrequent during the neonatal period with a reported incidence of 1–3/1000 admissions in NICU [81].

The pathogen most frequently isolated from infants with osteomyelitis or osteoarthritis is *S. aureus*. Other organisms include *C. albicans*, *E. coli*, GBS and CONS [80, 81]. Wong et al [81] studied 94 infants aged 0–3 months and investigated for osteomyelitis: in 28 out of 94 a microorganism was isolated, including methicillin-sensitive *S. aureus* in 16 cases, methicillin-resistant *S. aureus* in seven cases, *E. coli* in three cases, and GBS in two cases.

Organisms may reach the bones by hematogenous spread, which seems to be the most frequent route of infection in neonates, particularly those with vascular catheters. Less often, infection of the skin and tissues surrounding the bones are the source of infection [80].

During the neonatal period, osteomyelitis is frequently associated with arthritis. This association is explained by the vascular supply to the epiphysis and metaphysis in the newborn. In adults and older children, the blood supply for each structure is provided by separate vascular networks, while in the neonate before ossification, epiphyseal cartilage growth depends on metaphyseal vessels. Through these vessels, infection reaches the epiphysis and the joint causing septic arthritis [80].

Risk factors for osteomyelitis include preterm birth, the coexistence of a UTI or neonatal respiratory distress, and presence of vascular catheters [80, 82]. Wong et al [81] reported 30 infants with osteomyelitis, of whom 17 were mechanically ventilated preterm neonates, and four were term neonates undergoing intensive care.

Clinical suspicion of osteomyelitis must be confirmed by laboratory and radiological tests. During the early stages, clinical signs may be non-specific but swelling or immobility of the limb are common. Physical examination is often characterized by functional limitation of the involved limb, pain during passive movements, joint immobility, swelling, edema and soft tissue inflammation. General signs of infection include poor feeding and/or irritability; fever and abnormal inflammatory markers are not always present in the early stages. In a study of 25 newborn infants, Narang et al [83] reported functional limitation of the involved limb (64%) and local swelling (60%) as the most frequent clinical findings at the time of onset of ostemyelitis; multi articular involvement was reported in eight cases (32%). Hip (48%) and knee (48%) were most frequently involved. Wong et al [81] reported that osteomyelitis was multifocal in 40% of cases and associated with septic arthritis in 47%. In their cohort, fever was present in about 10% of cases.

Septic arthritis is a medical emergency. The destruction of the epiphysis by the infectious process and avascular necrosis caused by compression of the joint by the inflammatory exudate may result in a long-term, severe, functional limitation of the involved joint. The prognosis depends on the rapidity of the diagnosis and institution of appropriate treatment. Osteomyelitis should be suspected in every neonate with local pain, functional limb limitation, or systemic clinical signs without a known source of infection [80].

Microbiological confirmation to guide the choice of antibiotics or to change treatment in the case of lack of therapeutic response is desirable but only available in no more than 50–80% of cases [84]. Deshpande et al [85] reported 15 newborns with positive blood culture in only seven cases (47%) and synovial fluid culture in nine cases (60%). The isolation of a microorganism should be attempted by culture of blood, tip of the central venous catheter, and, if available, synovial fluid, including Gram staining.

X-rays of the involved limb may show nonspecific signs of osteomyelitis and septic arthritis such as edema of soft tissues and an increase of the articular space caused by the presence of a purulent exudates. Cortical osteolysis, a sign of bone necrosis, is seen only in the late stages of the disease. The ability of X-rays to detect lesions characteristic of osteomyelitis is limited in neonates because soft tissue edema and enlargement of the articular space are not always evident, and areas of osteolysis are rarely visible in the early phases, appearing as late as 10–21 days after the onset of symptoms [80, 84]. Despite these limitations, X-rays remain a relevant technique for the confirmation of osteomyelitis [86].

Ultrasound is a highly variable technique that depends on the experience of the physician; a negative result does not exclude osteomyelitis. However, it may be useful during the diagnostic workup of suspected osteomyelitis, showing soft tissue edema, subperiosteal abscesses or joint effusions [80, 87].

Bone scintigraphy with 99m-technetium enables diagnosis in 80–100% of cases. It has high sensitivity in detecting single or multiple foci of infection during the first 48–72 h after the onset of signs. These are seen as hyperperfused areas (expression of inflammation) or as "cold spots" (in areas where edema has reduced vascularization) [84]. However, in the neonatal period this technique has a lower diagnostic value than in older children and its use should be limited to neonates with a negative radiograph and clinical evidence of osteomyelitis.

Computerised tomography enables early detection of necrosis and periosteal reaction and may be useful in the diagnosis of osteomyelitis of the bones of the skull (e.g., after infection of a cephaloematoma). MRI may show abscesses and periosteal involvement, but may require sedation of the infant [86].

If a purulent synovial effusion is suspected, puncture should be always performed to reduce pressure within the joint and enable appropriate microbiological investigations.

Success of treatment depends on the appropriateness of antibiotics and the removal of the purulent effusion. The empirical antibiotic combination should take account of known likely etiologic agents: *S. aureus*, GBS, and Gram-negative bacteria. Intravenous vancomycin together with an aminoglycoside or a third generation cephalosporin (usually cephotaxime) are suitable options.

Therapy should be modified on the basis of the isolated organism and its sensitivity spectrum. If no microorganism is isolated, the initial empirical treatment should be continued until there is clinical improvement. Lack of response to initial antibiotic therapy should raise the suspicion of antibiotic resistance or the presence of an abscess, to be investigated by appropriate imaging.

Antibiotic therapy should be continued for at least 4–6 weeks or longer and until the clinical condition, radiological and laboratory investigations indicate resolution. Courses shorter than 3 weeks are associated with a high risk of relapse.

Estimates of the frequency of residual complications following osteomyelitis during the neonatal period vary from 6 to 50%. They are due to bone growth abnormalities, asymmetric limb size, gait defects and pathologic fractures [84, 85]. Factors affecting prognosis include the delay between the onset of symptoms and start of antibiotic treatment, the duration of antibiotics, and which joint is affected [84, 85]. The risk for bone deformities is highest for the hip and knee and diagnostic delays longer than 3–4 days [86].

116.2.8 Miscellaneous

116.2.8.1 Ophthalmia Neonatorum

Ophthalmia neonatorum (neonatal conjunctivitis) is the term for any purulent conjunctivitis starting during the first 28 days of life [88, 89]. It may have infectious and non infectious etiologies. Non infectious conjunctivitis is mainly chemical

conjunctivitis following prophylaxis of ophthalmia neonatorum with 1–2% silver nitrate. Infectious causes were identified by Amini et al [88], who reported 198 neonates with conjunctivitis. Bacteria included *S. aureus* (31%), *E. coli* (23%), *S. epidermis* (22%), *Klebsiella* (10%), *N. gonorrhoeae* (3%), *C. trachomatis* (2%), and *Pseudomonas aeruginosa* (2%). Viruses have also be implicated [90–92]. In developed countries, the most frequently isolated pathogen is *C. trachomatis*, with a reported incidence in the USA of 8.2/1000 live births [88, 93]. In developing countries, the most frequently isolated organisms are *S. aureus* and *C. trachomatis* [88, 89].

The clinical picture, regardless of etiology, is characterized by hyperemia of the conjunctivae, swelling and hyperemia of eyelids, and purulent conjunctival exudate [94]. Although the disease may be benign if treated promptly, neglected neonatal conjunctivitis may result in blindness.

After the introduction of Crede prophylaxis with 2% silver nitrate solution, first described in 1881 [95], there was a reduction in neonatal gonococcal conjunctivitis (the most important cause of blindness in the XVIII century) , and in infantile blindness. Since then, several agents have been proposed to combine wide antimicrobial protection with a reduced incidence of chemical conjunctivitis due to silver nitrate. These agents included 0.5% erythromycin, 1% tetracycline, and gentamicin [96, 97]. Isenberg et al [98] in a clinical trial on 3,117 newborn infants in Kenya, compared the use of 2.5% povidone-iodine with 1% silver nitrate or 0.5% erythromycin. The povidone-iodine solution was more effective than silver nitrate or erythromycin and less toxic. Richter et al [99] showed that 1.25% povidone-iodine solutions have no effect on thyroid function.

Although 0.5% erythromycin and 1% aureomycin are most used, 1% fusidic acid ophthalmic ointment is effective against the most frequent pathogens causing ophthalmia neonatorum (including *N. gonorrhoeae* and *C. trachomatis*), has a prolonged effect (for more than 12 hours) and is well tolerated.

116.2.8.2 Acute Otitis Media

Acute otitis media (AOM) is the presence of a purulent exudate in the middle ear, associated with clinical signs of acute disease. It may present as isolated disease, or in association with sepsis, meningitis, or pneumonia. Risk factors include the presence of meconium-stained amniotic fluid, the need for neonatal resuscitation, mechanical ventilation, prolonged tracheal intubation, and cleft palate. Breast fed neonates are less likely to be affected than formula fed infants [59].

Turner et al [100] studied 137 babies aged < 2 months with suspected AOM. At tympanocentesis, the most frequently isolated organism was *Streptococcus pneumoniae* (46%), followed by *Haemophilus influenzae* (34%), Group A *streptococcus* (10%), Gram-negative bacilli (7%), *Moraxella catarrhalis* (2%) and *Streptococcus faecalis* (1%). Mixed infections were observed in 20 cases: *S. pneumoniae* + *H. influenzae*, *H. influenzae* + *M. catarrhalis*, *S. pneumoniae* + *H. influenzae* + *M. catarrhalis*, and *Klebsiella* + *Enterobacter*.

Otitis presenting duuring first weeks is usually caused by the same organisms as neonatal sepsis (*S. aureus*, GBS and Gram-negative bacilli). By contrast, otitis presenting after the first 2 weeks in neonates without associated diseases is usually community acquired and frequently caused by *S. pneumoniae* [59].

The diagnosis of AOM is based on physical examination. Non specific signs, such as poor feeding and irritability may be the first clinical signs, with or without purulent exudate in the external ear. In the study by Turner et al [100], presentation was with fever (70%), conjunctivitis (46%), respiratory distress (27%), lower respiratory tract infections (13%), vomiting (4%), diarrhea (4%). Both ears were affected in 45% of infants. At otoscopic examination, there is hyperemia and bulging of the tympanic membrane, which is often cloudy with impaired mobility, and an air-fluid level or bubble visible in the middle ear. Tympanocentesis, when indicated, may decompress the middle ear and allows culture of the purulent exudate.

The presence of fever at presentation is not reliably predictive of increased risk of progression to sepsis [100, 101] Treatment should take account of the baby's age and possible pathogens.

The initial antibiotic treatment should be ampicillin in combination with an aminoglycoside or a third generation cephalosporin for 10 days for infants aged < 2 weeks or those who have clinical signs of systemic infection or who are hospitalized. For non-hospitalized neonates aged > 2 weeks and without associated diseases, the most probable infecting agent is *S. pneumoniae* and the first line antiobiotic is amoxicillin for at least 10 days [59].

116.2.8.3 Omphalitis

Omphalitis is infection of the umbilical cord. The incidence varies from 0.7% in developed countries and 6.2% in developing countries [102]. In a recent study by Mullany et al in Nepal on 17,198 infants [103], the incidence of omphalitis was 5.5%. Risk factors include prolonged labor, prolonged rupture of membranes, maternal infections, prematurity, low birth weight at birth, and the presence of umbilical cord catheters [102]. Mullany et al reported an increased risk for infants whose umbilical cord was disinfected with non hygienic solutions, and found that good hand hygiene of health care workers was protective [103].

Most frequently isolated organisms include *S. aureus*, *S. epidermidis*, Group A and Group B streptococci, *E. coli*, *Klebsiella*, *Pseudomonas*, *C. difficile* and anaerobic bacteria. In developing countries, *C. tetani* remains an important pathogen for omphalitis [102, 103].

Omphalitis is characterized by edema and hyperemia of the periumbilical skin, with or without purulent exudate. The

infectious process may progress deeper in soft tissues causing cellulitis and lymphangitis of the abdominal wall, sometimes involving the subcutaneous fat and deeper layers [102, 103].

Although omphalitis is a localized disease, it may progress to systemic disease if untreated. After infection of the periumbilical skin, dissemination of the infection may occur if there is umbilical vessel involvement. The most frequent complications of omphalitis are sepsis, necrotizing fasciitis, abscesses (of pelvis, retroperitoneum, skin, liver), peritonitis and hepatic vein thrombosis.

Prevention of omphalitis using 4% chlorexidine as first choice disinfectant has been recently evaluated by Mullany et al [104]: a randomized clinical trial compared the use of 4% chlorexidine (4,934 infants) with cleansing with soap and water (5,107 infants) or dry cord care (5,082 infants), and found a reduction of infections and of neonatal mortality in the chlorexidine group. Severe omphalitis requires urgent intravenous antibiotic treatment effective against staphylococci, streptococci and Gram-negative bacteria.

116.2.8.4 Impetigo Bullosa and Staphylococcal Scalded Skin Syndrome

Impetigo bullosa is a disease characterized by friable non-erythematous cutaneous vesicles, filled with a yellowish fluid, usually located in the periumbilical skin and the folds of the skin in the axialla or neck. It is caused by *S. aureus*, and has a benign course. The skin lesions are focal, but wider skin dissemination has been reported. Treatment is based on topical skin disinfectants, but systemic antibiotics are needed in the case of resistance to local disinfection.

Staphylococcal scalded skin syndrome (SSSS, previously known as Ritter's disease) is a disease characterized by cutaneous desquamation after light rubbing (Nikolsky's sign) that involves extensive areas of the skin. It is an exfoliative process indistinguishable from toxic epidermal necrolysis (TEN). The difference between the two clinical pictures is in the etiology: SSSS is caused by *S. aureus*, while TEN refers to cutaneous exfoliation caused by non-infectious agents or by infectious agents other than *S. aureus*.

The pathogenesis of SSSS is linked to the ability of some strains of *S. aureus* to produce exfoliative toxins A and B, which detach the stratum granulosum from deeper layers of the skin, leading to desquamation and rash, usually without fever, systemic symptoms, or bacteremia [105].

Pending the results of microbiological tests, the differential diagnosis between SSSS and TEN is by cytology of a smear from a swab of the detached skin: big epithelial cells with small nuclei and lack of inflammatory cells can be observed in SSSS, while cuboidal cells with large nuclei and of inflammatory cells can be observed TEN [10].

Treatment options for SSSS are β-lactam-resistant penicillins (oxacillin, flucloxacillin) or vancomycin, while corticosteroids, contraindicated for SSSS, may be used for TEN.

116.2.8.5 Neonatal Toxic Shock Syndrome-like Exanthematous Disease

In adults, the toxic shock syndrome toxin-1 (TSST-1) is caused by strains of *S. aureus*. A fulminant clinical picture is characterized by fever, hypotension, macular cutaneous rash associated with desquamation of the skin and multiorgan dysfunction. Sporadic neonatal cases have been reported.

Takahashi et al, in 1998 [106] reported 20 newborn infants with a clinical picture characterized by rash (appearing between 2 and 5 days of life) without desquamation and lasting 2–3 days. Associated thrombocytopenia at the time of the rash is sometimes preceded by fever lasting 1 day. All were colonized by a strain of methicillin-resistant *S. aureus* (MRSA) producing TSST-1. This clinical picture was described as a new nosologic entity and defined neonatal toxic shock syndrome-like exanthematous disease (NTED). The pathogenic mechanism seems to be linked to the ability of TSST-1 to act as a superantigen expanding the T cell compartment. High maternal antibody titers (anti-TSST-1 IgG) appear to be protective for NTED. Recently the same authors performed a case-control study of NTED in Japanese neonatal intensive care units and summarized the clinical findings of 540 patients. The number of NTED patients decreased over the 5-year period from 2000 to 2005 although there were more than 100 patients with NTED in Japan in 2005. Most patients recovered within 5 days of the onset of the exanthematous process without active treatment, but two preterm infants died during the recovery phase [106].

116.2.8.6 Noma Neonatorum

Noma neonatorum is a rare gangrenous process, frequently associated with bacteremia, involving the nose, mouth, eyelids, and perineum [107]. It mainly affects low birth weight or preterm infants, has a fulminant course, and a high mortality within the first 1-3 days after presentation. In most cases reported in the literature, there was an association with the isolation of *P. aeruginosa*. Treatment is based on antibiotics against *P. aeruginosa*.

116.2.8.7 Neonatal Tetanus

Neonatal tetanus is a severe condition, although rare in developed countries. It is caused by *Clostridium tetani*, which is a Gram-positive, obligate anaerobic, rod-shaped, spore-forming bacterium. Because it is an obligate anaerobe, localization of *C. tetani* in the periumbilical region may be prevented by disinfection of the umbilical cord with H_2O_2.

Prevention is by maternal tetanus immunization and neonates born to mothers with anti-tetanus toxin antibodies are protected from the disease by transplacental transfer of specific IgG. Aseptic obstetric and neonatal practices are also vital.

The time of incubation between inoculation of the spores and onset of symptoms varies from 3 to 21 days. About 90% of neonates with tetanus develop the disease during the first 3–14 days of life (mostly between days 6 and 8). The baby becomes febrile and presents with difficulty in opening the mouth for suction. There is muscle rigidity and spasms extending progressively to the chest wall and limbs and to the diaphragm, leading to respiratory failure. Laryngeal or glottal spasm may lead to airway obstruction or aspiration pneumonia. Episodes of cyanosis and apnea are common in uncontrolled severe disease. Before mechanical ventilation and the availability of effective agents to control muscle spasm, mortality was mainly because of respiratory failure. Autonomic dysfunction may lead to hypertension or hypotension, tachycardia, bradycardia, and arrhythmias that can result in life-threatening hemodynamic instability and cardiac arrest [108].

Neonatal tetanus is an emergency. Therapy consists of the use of antibiotics (penicillin), hyperimmune anti-tetanus toxin immunoglobulin (500 UI i.m.), and benzodiazepines (intravenous diazepam, 0.3–0.5 mg/kg/dose, repeatable). Endotracheal intubation and mechanical ventilation may be necessary to treat respiratory failure. The prognosis is characterized by a high mortality, but immediate and effective intensive care may reduce mortality to less than 20% [108].

116.3 Fungal Infections

The great majority of fungal infections in the neonate are caused by *Candida* spp, and less frequently by *Malassezia* spp. *Candida* is a commensal organism that usually colonizes the skin and mucous membranes of the gastrointestinal tract and respiratory airways. In the immunocompromised host it behaves as a pathogen, by invading host tissues and entering the circulation.

About 10% of term neonates and 27–63% VLBW neonates admitted to NICUs during the first weeks of life are colonized by fungi [13]. In a study conducted in six NICUs between 1993 and 1995 on 2,847 neonates by Rangel-Frausto et al, the incidence of sepsis caused by *Candida* was 12.3/1000 admissions (0.64/1000 patients-days); reported species were *C. albicans* (63%), *C. parapsilosis* (29%), *C. glabrata* (6%) and other species (3%: *C. tropicalis*, *C. kruse*, *C. lusitaniae*, *C. guilliermondii*, *C. dubliniensis*) [109].

In a survey conducted in 27 NICUs (20,565 neonates) in Spain, 118 babies (0.57%) had a systemic infection caused by *Candida*. *Candida* spp. were isolated from the blood culture of 79 neonates, from urine cultures of 33, and from the CSF of four. The frequency of infection was significantly higher in VLBW infants (4.8%) than in infants with birth weight >1500 g (0.2%) ($P < 0.001$) [110].

Candida may be acquired by horizontal or, less frequently, vertical transmission. Horizontal transmission occurs mainly through the contaminated hands of health care workers. In a multicenter study involving six NICUs [111], *C. albicans* was isolated from the hands of 5% of health care workers, and *C. parapsilosis* from 19%. 486 neonates out of 2,157 (23%) were colonized by *Candida*: 299 (14%) by *C. albicans*, 151 (7%) by *C. parapsilosis* and 74 (3%) by other species. Other sources of infection may be parenteral nutrition solutions or contaminated infusion circuits. Rarely, the reservoir is the environment.

Invasive *Candida* infections are generally preceded by colonization. In a multicenter study of 2847 neonates [112], candidemia was preceded by colonization in 15/35 (43%) cases.

Both neonatal risk factors and the virulence of the microorganism are implicated in the pathogenesis of invasive *Candida* infections. The most important neonatal risk factors for nosocomial candidiasis are prolonged administration of broad spectrum antibiotics (courses longer than 21 days), extreme prematurity (gestational age at birth < 28 weeks) because of impaired humoral and cellular imunity, and the presence of venous catheters (especially if prolonged). Additionally, Saiman et al [112] found that total parenteral nutrition and intravenous lipid solutions, administration of H2-blockers, , endotracheal intubation, disseminated intravascular coagulation and prolonged hospital stay were also significant risk factors for invasive *Candida* infections ($P < 0.05$). All types of catheter (vascular, vesical, peritoneal catheters, ventriculoperitoneal and thoracic drains, endotracheal tubes) favor adhesion, growth and penetration of microorganisms [113]. Feja et al [114] recently reported associations between invasive *Candida* infections and gastrointestinal tract diseases (NEC and focal intestinal perforation; OR = 4.57) and previous bacterial sepsis (OR = 8.02). Benjamin et al, studying 4,579 extremely low birth weight infants (< 1000 g; ELBW), found that delay in starting enteral nutrition was an added risk factor. Steroid therapy, hyperglycemia, neutropenia, and abdominal or cardiac surgery are additional risk factors [113].

The virulence of the microorganism is linked to its ability to form hyphae (that permit tissue invasion), to the infecting species (*C. albicans* is the most virulent), to the expression of adhesins, and to the ability to form a biofilm that protects the microorganism from the host immune response and from exposure to antifungal drugs. Mixed biofilms (*Candida* together with CONS) are not infrequent. Adam et al [115] found that the slime produced *in vitro* by *S. epidermidis* inhibits the penetration of fluconazole, and that the slime produced by *C. albicans* protects staphylococci from the action of vancomycin.

Once the cutaneous and mucous surfaces are colonized, *Candida* may invade tissues locally, leading to parenchymal destruction, sometimes with the formation of abscesses, which are difficult to eradicate. Circulatory involvement may lead to the development of sepsis with end-organ dissemination (endocardium, kidney, central nervous system, bone) [116].

There are three clinical pictures: congenital candidiasis, mucocutaneous candidiasis, and fungal sepsis.

Congenital candidiasis is the congenital infection of the fetus by *Candida* species in the presence of *Candida*

chorioamnionitis. It is a rare event, accounting for 0.8% of cases of chorioamnionitis [13, 117]. In about 25% of cases it occurs in women with intrauterine devices and heavily colonized by *Candida* [13]. Affected infants present with pustules, vesicles, skin abscesses, and an erythematous maculopapular rash on the trunk and extremities that sometimes evolves to desquamation [118]. Histologic evaluation of the umbilical cord and placenta shows microabscesses, hyphae, and granulomas, confirming the diagnosis [13]. Clinical features and evolution of the disease are generally more severe in the preterm infant than in those born at term. Darmstadt et al, reviewing the literature of infants with congenital candidiasis, found that those with birth weight < 1000 g were at higher risk of systemic infection than those > 1000 g (67% *vs* 10%) [118]. Preterm neonates may present with organ involvement (pneumonia, disseminated dermatitis, hyphae in urine, blood and CSF).

Mucocutaneous candidiasis is the most common manifestation of the disease. It usually presents during the second or third week of life as a diffuse erythematous rash affecting the napkin area and white patches on the oral mucosa, gingivae and tongues. As the disease progresses, the skin rash becomes more widespread, affecting the abdominal skin and chest. The rash may be papular or pustulous or associated with cutaneous desquamation [119].

Cutaneous involvement may precede systemic spread [120]. The clinical manifestations of fungal sepsis are similar to those of bacterial sepsis. Thrombocytopenia is nonspecific: Guida et al [121] found that 84% of infants with fungal sepsis, 75% of infants with Gram-negative sepsis, and 48% of infants with Gram-positive sepsis had platelet counts < 100,000/mm^3.

Frequently, at the time of diagnosis, the infection is already disseminated to organs. *Candida* spp. may cause organ infections as endocarditis, meningitis, endophthalmitis and chorioretinitis, dermatitis, peritonitis, osteomyelitis, and septic arthritis, and may form abscesses in the central nervous system, kidneys, liver, spleen, and gut. In a recent meta-analysis, the rate of positive urine cultures was 61% among neonates with candidemia, but ultrasound findings of renal involvement were found in only 5% of cases. The rate of meningitis was 15%, cerebral abscesses or ventriculitis were found in 4% of cases, endocarditis in 5% and endophthalmitis in 3% [122].

Urine and CSF cultures, together with blood cultures, should always be performed in infants with suspected *Candida* sepsis. Microscopic examination of the urine may reveal hyphae and ultrasound may show evidence of renal tract involvement. Other imaging studies may include cardiac and cerebral ultrasound and ophthalmologic examination if ophthalmitis is suspected [113].

The identification of the gene coding for 18S rRNA by PCR is under investigation. This gene is present in the genome of almost all clinically relevant fungal species is absent in the human genome. Löffler et al [123] compared the identification of the 18S rRNA gene with traditional culture methods for the diagnosis of fungal infection in 600 samples from febrile and neutropenic adult patients with neoplastic diseases. Compared to traditional cultures, the PCR showed 100% sensitivity and 98% specificity for fungal infections.

Sepsis by *Candida* is associated with increased mortality and morbidity. In a study by the Neonatal Research Network [6], the mortality rate for VLBW neonates with fungal sepsis was three-fold higher than for those without.

Sepsis caused by *C. albicans* is associated with a higher mortality compared to sepsis caused by other *Candida* species. In a study of 45 newborn infants, Faix et al [124] found that deaths attributable to infection by *Candida* were 7/29 in infants with infection by *C. albicans* and 0/16 in infants with infection by *C. parapsilosis* (P = 0.034). In the study by the Neonatal Research Network, the mortality rate for VLBW infants with *C. albicans* infection was 43.9% compared with 15.9% for *C. parapsilosis* [6].

In a study conducted between 1988 and 1996 on ELBW neonates with sepsis and/or meningoencephalitis caused by *Candida*, the outcome at 2 years corrected age was compared with a cohort of ELBW neonates without *Candida* infection. Case fatality rate was similar. However, all survivors in the *Candida* group had chronic lung disease at discharge, compared with 33% of the control cases (P = 0.0001). There was a high incidence of periventricular leukomalacia (26% *vs* 12%, P = 0.06) and increased severe retinopathy of prematurity (22% *vs* 9%, P = 0.04). 60% of the *Candida* group had adverse neurologic outcomes at 2 years corrected age compared with 35% in the control group, and 41 *vs* 12% had severe disabilities (P = 0.005) [125].

The first line antifungal drug for the treatment of fungal sepsis is amphotericin B, which is generally well tolerated by the neonate. The dosage is 0.1 mg/kg/day administered intravenously in a single daily dose in 2 hours. The dose may be progressively increased to a maximum of 1 mg/kg/day. In some studies on adult patients, levels achieved in the CSF are about 5–10% of plasma levels, but in a small study of 13 preterm infants, CSF levels were 40–90% of plasma levels.

Liposomal amphotericin B is less toxic and can be administered at higher dosages. Amphoteriocin B is potentially nephrotoxic, and if renal function is compromised, the liposomal formulation of amphotericin B is indicated. Liposomal amphotericin B is prescribed at 1–5 mg/kg/day. The effectiveness and safety of a dose of 5–7 mg/kg/day of liposomal amphotericin B was studied prospectively by Juster-Reicher et al [126] in 2003. In 41 cases of systemic candidiasis, the authors found it to be highly effective (recovery in 95% of cases) without side effects except for one infant who had a transient increase in liver enzymes. The time required for eradication was 10.9 ± 4.8 days for infants who had previously received other antifungal drugs and 8.7 + 4.5 days for infants in whom liposomal amphotericin B was used as the treatment of first choice. The recommended dose for intravenous liposomal amphotericin B is 1 mg/kg/day administered over 2 hours, increasing progressively to a maximum of 5 mg/kg/day.

Fluconazole is the most studied azole in the neonatal age range. Its use for the prevention of fungal infections in high

risk neonates has been evaluated by several studies, but amphotericin B is the first choice for treatment of an infection. Several studies recommend intravenous 5-fluorocytosine in combination with amphotericin B for severe fungal infections. 5-fluorocytosine achieves high CSF concentrations, and is recommended for fungal meningitis. However, it should never be used as a single antifungal because of the frequent emergence of resistant strains. The recommended dose is 50–150 mg/kg/day intravenously over 15 minutes in 4 daily doses.

A new class of drugs, the echinocandins, act through noncompetitive inhibition of the synthesis of the 1,3-β-glucan in the cellular wall and have been used in adults. Studies to evaluate their effectiveness and safety in the neonate are ongoing.

The duration of antifungal treatment should be decided on the basis of clinical and microbiological factors: the fungal species, the presence of a central venous catheter, which should always be removed in infants with fungal infections, the number of positive cultures, CSF cell count and chemistry, imaging (echocardiography, renal ultrasound, ophthalmologic examination and imaging of the central nervous system). In infants with systemic fungal infections, intravenous treatment should be prolonged for at least 2–4 weeks after the last negative blood culture. Longer courses may be needed for meningitis and end-organ dissemination. Starting empirical antifungal treatment before the availability of culture results has not been the subject of prospective studies. Some high risk populations, such as ELBW neonates with sepsis and risk factors for fungal infections (presence of a central venous catheter, endotracheal tube, thrombocytopenia, prolonged treatment with cephalosporins or carbapenems, gestational age < 28 weeks) may be helped by empirical treatment while awaiting culture results, especially if antibiotics are not apparently effective.

116.4 Prevention of Early- and Late-Onset Infections

116.4.1 Prevention of Early-Onset Group B Streptococcal Infection

116.4.1.1 Intrapartum Antibiotic Prophylaxis (IAP)

Intrapartum antibiotic prophylaxis (IAP) is currently the most important preventive measure against early-onset group B streptococcal (GBS) disease. There are two strategies. One is based on universal prenatal screening while the other is based on the presence of intrapartum risk factors. The former was proposed by the Centers for Disease Control and Prevention (CDC) [127]. The latter is based on the presence of intrapartum risk factors and is recommended by the UK Royal College of Obstetricians and Gynaecologists (RCOG) [128]. Several studies, in particular a case-control study of 5144 births published by Schrag et al to compare the two strategies showed that the screening-based approach was better than the risk-based approach [129]. The introduction and the widespread use of IAP and especially the implementation of guidelines issued by the CDC have led to a substantial reduction in the incidence of early-onset GBS disease. A recent retrospective study demonstrated that screening for GBS before delivery increased from 48% in 1998–1999 to 85% in 2003–2004 and women with an indication for IAP who received prophylaxis increased from 74 to 85% [130]. This has resulted in a reduction in the incidence of early-onset GBS disease in the USA from 1.7 to 0.35/1000 live births [131]. The drug of choice for IAP is penicillin G i.v. (5 million units followed by 2.5 million units every 4 hours until birth). This drug is not generally available and an alternative is intravenous ampicillin (2 g followed by 1 g every 4 hours until birth) [132].

The screening-based method requires a vaginal-rectal swab to be performed on all pregnant women at 35–37 weeks of gestation and the administration of *intrapartum* antibiotics to colonized women at the time of labor.

The 2010 CDC guidelines provide criteria for identification of candidates to receive intrapartum antibiotic prophylaxis to prevent early-onset GBS disease (Table 116.4). The following are key components of the screening strategy [127]:

- Women with GBS isolated from the urine at any time during the current pregnancy or who had a previous infant with invasive GBS disease should receive intrapartum antibiotic prophylaxis and do not need third trimester screening for GBS colonization. Women with symptomatic or asymptomatic GBS urinary tract infection detected during pregnancy should be treated according to current standards of care for urinary tract infection during pregnancy and should receive intrapartum antibiotic prophylaxis to prevent early-onset GBS disease.
- All other pregnant women should be screened at 35–37 weeks' gestation for vaginal and rectal GBS colonization.
- At the time of labor or rupture of membranes, intrapartum antibiotic prophylaxis should be given to all pregnant women who tested positive for GBS colonization, except in the instance of cesarean delivery performed before onset of labor on a woman with intact amniotic membranes.
- For circumstances in which screening results are not available at the time of labor and delivery, intrapartum antibiotic prophylaxis should be given to women who are <37 weeks and 0 days' gestation, have a duration of membrane rupture ≥18 hours, or have a temperature of ≥100.4°F (≥38.0°C).
- In the absence of GBS urinary tract infection, antimicrobial agents should not be used before the intrapartum period to eradicate GBS genitorectal colonization, because such treatment is not effective in eliminating carriage or preventing neonatal disease and can cause adverse consequences.
- Intrapartum antibiotic prophylaxis to prevent early-onset GBS disease is not recommended as a routine practice for cesarean deliveries performed before labor onset on women with intact amniotic membranes, regardless of the GBS colonization status of the woman or the gestational

Table 116.4 Indications for intrapartum antimicrobial prophylaxis (IAP) to prevent early-onset GBS disease. Modified from [127]

Intrapartum GBS prophylaxis indicated	Intrapartum GBS prophylaxis not indicated
• Previous infant with invasive GBS disease	• Colonization with GBS during a previous pregnancy (unless an indication for GBS prophylaxis is present for current pregnancy)
• GBS bacteriuria during any trimester of the current pregnancy[1]	• GBS bacteriuria during previous pregnancy (unless an indication for GBS prophylaxis is present for current pregnancy)
• Positive GBS vaginal-rectal screening culture in late gestation[2] during current pregnancy[1]	• Negative GBS vaginal-rectal screening culture in late gestation[2] regardless of intrapartum risk factors
• Unknown GBS status at the onset of labor (culture notdone, incomplete, or results unknown) and any of the following: – Delivery at <37 weeks' gestation – Amniotic membrane rupture ≥18 hours – Intrapartum temperature ≥100.4°F (≥38.0°C)[3] – Intrapartum NAAT[4] positive for GBS	• Cesarean delivery performed before onset of labor on a woman with intact amniotic membranes, regardless of GBS colonization status or gestational age

[1] Intrapartum antibiotic prophylaxis is not indicated in this circumstance if a cesarean delivery is performed before onset of labor on a woman with intact amniotic membranes.
[2] Optimal timing for prenatal GBS screening is at 35–37 weeks' gestation.
[3] If amnionitis is suspected, broad-spectrum antibiotic therapy that includes an agent known to be active against GBS should replace GBS prophylaxis.
[4] Nucleic acid amplification tests (NAAT) testing for GBS is optional and might not be available in all settings. If intrapartum NAAT is negative for GBS but any other intrapartum risk factor (delivery at <37 weeks' gestation, amniotic membrane rupture at ≥18 hours, or temperature ≥100.4°F [≥38.0°C]) is present, then intrapartum antibiotic prophylaxis is indicated.

age of the pregnancy. The use of perioperative prophylactic antibiotics to prevent infectious complications of cesarean delivery should not be altered or affected by GBS status. Women expected to undergo cesarean deliveries should undergo routine vaginal and rectal screening for GBS at 35–37 weeks' gestation because onset of labor or rupture of membranes can occur before the planned cesarean delivery, and under those circumstances GBS-colonized women should receive intrapartum antibiotic prophylaxis.

There are two main limitations to the universal screening-based strategy: a) the possibility that the woman delivers before vaginal swabs have been taken or before the results are available; b) the possibility of colonization between obtaining a negative swab and delivery. Yancey et al [133] showed that swabs performed 5 weeks before delivery predict colonization at delivery. A retrospective study reported negative screening results in 61% of GBS early onset disease, an unexpectedly high percentage. The authors suggest several reasons for false negative results: screening outside the 5 week window before delivery, errors in collecting and processing swabs and possible errors in the communication of results [130]. There is debate about the minimum duration of effective IAP. The CDC protocol recommends IAP administration at least 4 hours before delivery, but the RCOG recommends at least 2 hours.

In a prospective study of 21 women treated with ampicillin before elective cesarean section, Colombo et al [134] demonstrated bactericidal levels of ampicillin in cord blood 30 minutes after ampicillin administration. The cord blood ampicillin concentration is higher than the maternal blood concentration and both continue to be above minimal bactericidal concentrations 5–6 hours after administration. Ampicillin levels decreased more rapidly in the maternal than the fetal compartments, suggesting decreased clearance of ampicillin by the newborns. Lijoi et al observed significantly lower colonization in babies of mothers given IAP more than 4 h before delivery (3.7%) than those born to mothers who received IAP closer to the time of birth (12.3%) [135].

In conclusion, beta-lactam antibiotics for GBS prophylaxis administered for ≥4 hours before delivery have been found to be highly effective at preventing vertical transmission of GBS and early-onset GBS disease. Shorter durations of appropriate antibiotics might provide some protection.

116.4.1.2 Side Effects of IAP

The striking success of this preventive strategy, however, has raised the issue of possible side effects resulting from the adoption of intrapartum antibiotic prophylaxis on a large scale.

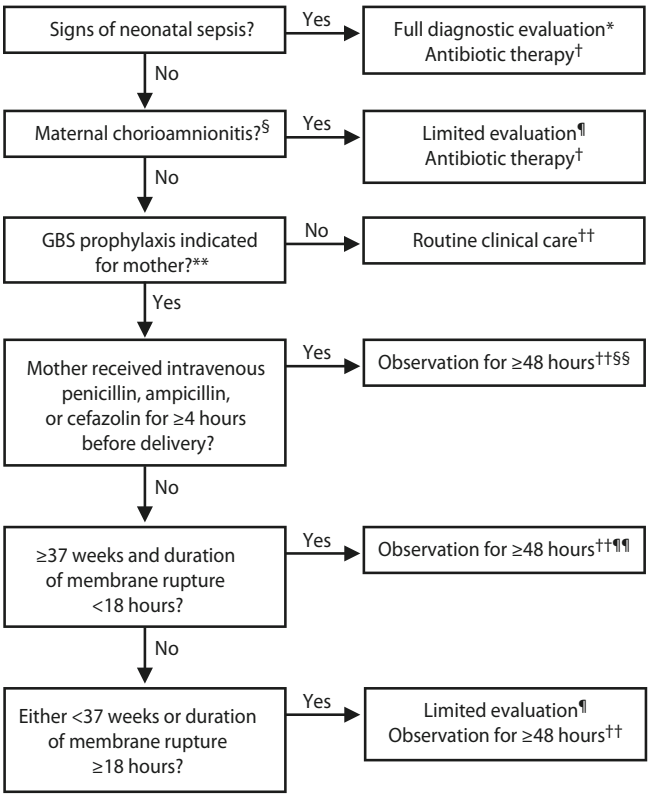
Van Dyke et al in a study of 7691 births found no cases of anaphylaxis after IAP [130].

The effect of IAP on neonatal infections by multi-resistant microorganisms other than GBS has been considered by Moore et al [136] who reviewed 412 articles and explored potential associations between IAP use and changes in the causes of early-onset sepsis. They found: a) a decreased incidence of GBS early-onset infections and some other bacteria, b) an increase in non-GBS or antimicrobial-resistant early-onset sepsis amongst preterm babies, and c) no change in the incidence of early onset disease by other organisms. These conclusions led Baltimore to suggest that the risks and benefits of IAP should be monitored in preterm and VLBW infants [137]. GBS resistance to erythromycin and clindamycin has been described but never to date to penicillin, ampicillin, cefazolin and vancomycin. In a review of 21 studies published between 1996 and 2006, Barcaite et al [138] reported no GBS strains resistant

to penicillin, ampicillin, cafazolin and vancomycin. Between 4 and 21% of GBS strains were resistant to erythromycin and between 3 and 20% were resistant to clindamycin [139–144].

116.4.1.3 Neonatal Management

Any baby who is unwell with clinical signs compatible with infection must be investigated and treated with antibiotics regardless of any risk factor that may or may not be present. In 2010 the CDC has provided an algorithm for secondary prevention of early-onset GBS disease among newborns (Fig. 116.2) [127].



* Full diagnostic evaluation includes a blood culture, a complete blood count (CBC) including white blood cell differential and platelet counts, chest radiograph (if respiratory abnormalities are present), and lumbar puncture (if patient is stable enough to tolerate procedure and sepsis is suspected).
† Antibiotic therapy should be directed toward the most common causes of neonatal sepsis, including intravenous ampicillin for GBS and coverage for other organisms (including *Escherichia coli* and other gram-negative pathogens) and should take into account local antibiotic resistance patterns.
§ Consultation with obstetric providers is important to determine the level of clinical suspicion for chorioamnionitis. Chorioamnionitis is diagnosed clinically and some of the signs are nonspecific.
¶ Limited evaluation includes blood culture (at birth) and CBC with differential and platelets (at birth and/or at 6–12 hours of life).
** See Table 116.4 for indications for intrapartum GBS prophylaxis.
†† If signs of sepsis develop, a full diagnostic evaluation should be conducted and antibiotic therapy initiated.
§§ If ≥37 weeks' gestation, observation may occur at home after 24 hours if other discharge criteria have been met, access to medical care is readily available, and a person who is able to comply fully with instructions for home observation will be present. If any of these conditions is not met, the infant should be observed in the hospital for at least 48 hours and until discharge criteria are achieved.
¶¶ Some experts recommend a CBC with differential and platelets at age 6–12 hours.

Fig. 116.2 Algorithm for secondary prevention of early-onset GBS disease among newborns. Modified from [127]

Treatment of a healthy GBS colonized neonate can create resistant bacteria, does not eliminate colonization, and therefore, does not prevent possible late infection [145]. Superficial swabs in neonates during the first hours of life have not been found to be useful [146, 147]. Indeed, about 90% of early onset disease occurs during the first 24 hours of life when the results of the cultures are not available [146] and 99% of the colonized neonates do not become infected. Guidelines for the prevention of perinatal GBS disease are available at the website www.cdc.gov/groupbstrep/guidelines/guidelines.html.

116.4.2 Prevention of Nosocomial Infections

Neonatal nosocomial infections are late onset infections (appearing after the first 72 hours of life) in hospitalized infants [1]. The incidence of infections varies widely among NICUs, depending on environmental factors and on differences in clinical practice. A survey by the World Health Organization (WHO) reported that nosocomial infection rates between 7 and 19% in Europe, and 14% in the USA [148]. Immunological immaturity, the frequent invasive procedures and prolonged hospitalization all account for the high incidence of infection amongst neonates. Infections may prolong the time of hospitalization and increase mortality and morbidity.

Prevention of health care associated infections is based on strategies that aim at limiting susceptibility to pathogens by reducing risk factors while enhancing host defenses, at interrupting transmission of organisms by health care workers, and at promoting the judicious use of antimicrobials [149].

Strategies for the prevention of nosocomial infections include hand hygiene practices, prevention of central venous catheter-related bloodstream infections, appropriate use of antimicrobials for therapy and chemoprophylaxis, skin care and early enteral feeding with human milk.

116.4.2.1 Hand Hygiene

Since Ignaz Semmelweis demonstrated dramatic reductions in puerperal sepsis after instituting a hand washing regimen in 1847, hand hygiene has been known to reduce health care associated infections and is recommended by the CDC as the single most effective method of preventing the spread of nosocomial infections [150]. Hand washing is a simple, inexpensive procedure that prevents the transmission of infectious pathogens. However, hand hygiene compliance remains low among health care workers, with reported adherence rates ranging from 20 to 50% [151].

In a multicenter clinical trial by Pittet et al [152], an intense campaign to promote hand hygiene practices led to a

significant increase in compliance with protocols (from 48 to 66%, P < 0.001), and a concomitant decrease in the overall frequency of nosocomial infections (from 16.9 to 9.9%, P = 0.04) and of infections caused by MRSA (from 2.16 to 0.93 cases/10,000 patients-days, P < 0.001).

The choice of the best method of hand hygiene is still a matter of debate. Many authors agree that hand rubbing with alcohol-based products (mainly 2-propanol-based solutions and alcohol-based preparations with 0.5% chlorhexidine) is microbiologically more effective than traditional hand washing with soap and water. It seems to be the best method of increasing compliance, being faster and less irritating for the skin. Antiseptic hand rub solutions can be placed at the bedside of each patient [153].

Guidelines for hand hygiene by the CDC Healthcare Infection Control Practices Advisory Committee (HICPAC) [154] (http://www.cdc.gov/mmwr/PDF/rr/rr5116.pdf) recommend hand washing with antimicrobial or non-antimicrobial soap and water when hands are visibly soiled or contaminated with proteinaceous material, and hand decontamination with an alcohol-based hand rub if hands are not visibly soiled and before engaging in direct contact with patients. Additional recommendations include avoiding wearing artificial fingernails and rings, bracelets and watches when engaging in direct contact with patients.

116.4.3 Prevention of Central Venous Catheter-Related Infections

Strategies for the prevention of central venous catheter-related infections include: 1) precautions during insertion and management of the central venous catheter; 2) reduction of indwelling times; 3) central venous catheters removal after positive blood culture results.

Central venous catheter contamination and colonization may occur during insertion and line management [155]. Good hand hygiene combined with proper aseptic technique during catheter insertion and manipulation affords protection against infection. Standard aseptic techniques (caps, masks, sterile gowns, sterile gloves, and large sterile drapes) during insertion substantially reduce the incidence of catheter-related bloodstream infections compared with standard precautions (sterile gloves and small drapes) [156, 157]. Proper management of the central venous catheters should include the establishment of a sterile field and rubbing the hub vigorously with alcohol at the time of hub entry or disconnection [27], avoiding the use of multilumen catheters, blood withdrawal through the catheter, and daily assessment of the site of insertion and of the integrity of the dressing [158]. Recently, Garland et al [159] conducted a surveillance study during a randomized trial to assess the safety and efficacy of a prophylactic vancomycin-heparin catheter-lock solution for the prevention catheter-related bloodstream infections. Nosocomial bloodstream infection was identified in 23 of the 82 neonates in the cohort. Fifteen of these infections were considered catheter-related bloodstream infections, which were intraluminally acquired in 10 (67%) of 15 patients (isolation of the same organism from the hub and blood cultures but not from the tip of the catheter), extraluminally acquired in three (20%, isolation of the same organism from the tip of the catheter and from blood cultures), and indeterminate in two (13%). The authors emphasized the importance of strategies for preventing intraluminally acquired catheter-related bloodstream infections.

The US Department of Health and Human Services Centers for Disease Control recommend a 2% aqueous chlorhexidine gluconate solution for skin disinfection before vascular access [160]. Administration sets should be replaced every 48–72 hours; lipid-containing sets should be replaced every 24 hours [156].

Reducing the indwelling time of central venous catheters is the easiest way to reduce the risk of catheter-related infection. Several studies demonstrated a direct correlation between times of indwelling catheters and incidence of sepsis in newborn infants [155].

In a prospective study by the NICHD Neonatal Research Network on VLBW infants, peripherally inserted central venous catheters placed for < 7 days increased the risk of infection 1.9 times (P < 0.001), but the risk increased to 3.7 times (P < 0.001) for catheters in place for 22 or more days [6].

Central venous catheters should be promptly removed when blood cultures are positive. Benjamin et al found that the outcome for patients in whom the central catheter was not removed within 24 hours of bacterial organism identification was significantly worse (increased risk of infectious complications defined as the presence of end-organ damage, multiple positive blood cultures drawn at separate times within one episode of sepsis, or death) than for those whose catheters were removed promptly (8% *vs* 46%; OR = 9.8) [161]. In a retrospective study by Karlowicz et al, central venous catheter removal within 3 days of the first positive blood culture for *Candida* species was associated with a shorter duration of fungemia (3 *vs* 6 days, P=0.0002) and a reduced mortality rate (2 *vs* 19%, P = 0.008) [162].

Central venous catheters should be removed as soon as bacteremia and fungemia are detected. When blood cultures are positive for CONS, catheter sterilization may be attempted, but the catheter should be promptly removed if subsequent cultures are positive.

116.4.4 Antimicrobial Prophylaxis

116.4.4.1 Antibiotic Prophylaxis

There is no good evidence to support antibiotic prophylaxis for high risk infants. In a Cochrane meta-analysis, antibiotic

prophylaxis for infants with central venous catheters slightly reduced the incidence of sepsis but this effect was not associated with a reduction in mortality. There were no data on neurodevelopmental outcome and on the emergence of resistant bacterial strains and antibiotic prophylaxis cannot be routinely recommended [163].

A Cochrane meta-analysis of five clinical trials studying the use of prophylactic vancomycin for the prevention of central venous catheter-related infections [164] reported that prophylactic vancomycin in low doses reduced the incidence of sepsis but the authors concluded that routine prophylaxis with vancomycin should not be undertaken at present.

An alternative approach for prophylaxis of central venous catheter-related nosocomial infections is "antibiotic flush" or "antibiotic lock" prophylaxis. In a prospective randomized clinical trial [165], VLBW infants with a newly placed peripherally inserted central venous catheter were randomized to having the catheter locked 2 or 3 times daily for 20 or 60 minutes with heparinized normal saline or heparinized saline containing vancomycin 25 μg/mL. The incidence of catheter-related bloodstream infections was significantly higher in the control than in the treated group (17.8 *vs* 2.3 per 1000 catheter days) [166]. No vancomycin-resistant enterococci or CONS were recovered from any cultures.

116.4.4.2 Antifungal Prophylaxis

Oral antifungal prophylaxis has been considered by a Cochrane review and there is insufficient evidence to support the use of prophylactic oral antifungal agents in VLBW infants in the NICU [167].

Randomized studies have shown that prophylactic intravenous fluconazole lowered the risk of invasive fungal infections and colonization [168, 169] and does not select resistant *C. albicans* strains [170]. Although fluconazole prophylaxis seems effective and well tolerated, further studies are needed to better establish subpopulations among neonates admitted to the NICU who could benefit from the drug [169, 171].

116.4.4.3 Immune Prophylaxis

Several studies have evaluated the effectiveness of immunoglobulins in preventing nosocomial infections in the preterm infant. The overall conclusion is that the small reduction in nosocomial sepsis is insufficient to support the routine use of immunoglobulins [46, 172]. Some benefit has been demonstrated for high risk infants of birth weight <1000–1250 g treated with intravenous immunoglobulin but the incidence of sepsis in the control group was very high (15–20%) [14].

Recently, DeJonge et al evaluated INH-A21, a plasma-derived, donor-selected polyclonal antistaphylococcal human immunoglobulin but failed to reduce the incidence of staphylococcal late onset sepsis in premature infants [173]. Another antistaphylococcal immune globulin, directed against serotypes 5 and 8 of *S. aureus*, is at present under investigation.

116.4.5 Skin Care

The skin barrier is compromised in the neonate, particularly if preterm, because of a thinner stratum corneum (3–16 layers, depending on gestational age at birth), which is easily damaged by handling, adhesives, and alcohol and povidone iodine applications. A Cochrane review concluded that the prophylactic application of topical ointments increased the risk of CONS infection and any nosocomial infection and that this treatment should not be used routinely in preterm infants [174].

A trial of daily massage with sunflower seed oil or Aquaphor (petrolatum, mineral oil, mineral wax, lanolin alcohol) found no significant reduction in the risk of infection, although skin applications of sunflower seed oil gave some protection against nosocomial infections [79]. The question whether topical skin ointment should be used for VLBW infants is still debatable and further trials are needed.

116.4.6 Breast Milk, Probiotics and Lactoferrin

The age of introduction of enteral feeds and the administration of human milk affects the incidence of nosocomial sepsis. The incidence of infection is reduced by human milk compared to formula [40] and early enteral feeding is also beneficial [175]. Possible mechanisms include: 1) prevention of gastrointestinal atrophy; 2) prevention of intestinal bacterial contamination; 3) decreased use of total parenteral nutrition and intravenous devices; 4) enhancement of mucosal immunity [175].

Several factors present in breast milk have an anti-infectious activity, including immune globulins, cytokines, complement, fibronectin, enzymes (lysozyme, leukocyte enzymes), macrophages (~60%), neutrophils (~25%), lymphocytes (~10%).

Studies of probiotics to prevent nosocomial sepsis [176, 177] have reported conflicting data and larger multicenter trials are needed to assess the risks and benefits of probiotics [178]. Recently, Manzoni et al [179] established that bovine orally administered lactoferrin, a mammalian milk glycoprotein involved in innate immune host defenses, alone (100 mg/day) or in combination with the probiotic *Lactobacillus rhamnosus* GG (6x109 colony-forming units/day) from birth until day 30 of life (day 45 for neonates < 1000 g at birth) significantly reduces the incidence of a first episode of bacterial and fungal LONS in VLBW neonates (risk ratio for lactoferrin *vs* control groups: 0.34; 95% CI, 0.17–0.70; P = 0.002. Risk ratio for lactoferrin plus *Lactobacillus rhamnosus vs* control groups: 0.27; 95% CI, 0.12–0.60; P < 0.001) [179, 180].

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Neonatal Septic Shock

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117.1 Introduction

Group B *Streptococcus* (GBS) continues to be a leading infectious cause of neonatal morbidity and mortality in the United States [1]. Overall, the incidence of GBS early onset disease (EOD) over the last 3 decades has decreased from 1.7 to 0.4 per 1,000 live births, a 70% reduction [2]. This success has largely been attributed to the institution of intrapartum prophylaxis. Since implementation of Group B streptococcal (GBS) prophylaxis, there has been a reduction in GBS EOD from 5.9 to 1.7 per 1000 live births of infants weighing 401–1500 g, but a concomitant increase in the rate of *Escherichia coli* sepsis from 3.2 to 6.8 per 1000 live births [3].

Investigators used hospital discharge data (from approximately one quarter of the U.S. population) to estimate that 10% of infants and children with severe sepsis (bacterial or fungal infection plus organ failure) died in 1995 [4, 5]. The incidence of sepsis in the neonatal population was 3.06 per 1000 children. A decade later, in a follow-up study, the authors noted that the incidence of sepsis in the neonatal population had decreased to 2.22 per 1000 children. Despite the decline in the overall incidence of sepsis, one cohort that demonstrated a significant increase in the incidence of sepsis was the very low birth weight (VLBW) group, defined as less than 1500 g (26.5% *vs* 9.2% in 1995) [6]. Similar observations were made by Stoll and colleagues who published a series of epidemiologic evaluations of newborn sepsis/septicemia/septic shock using United States Vital Statistics as well as the National Institute of Child Health and Human Development sponsored newborn infection registry. Neonatal mortality steadily decreased over the past decade, but remained most prominent in low birth weight neonates, defined as less than 2500 g [7–9]. Low birth weight newborns with EOD are more likely to die than those without infection (37% *vs* 13%). Twenty one percent of low birth weight newborns also have one or more episodes of blood culture positive late onset disease (LOD) and are also more likely to die (18% *vs* 7%). In VLBW newborns with LOD, the common causative organisms were gram positive (70%), followed by gram negative (17.6%) and fungal (12.2%) organisms. In the same study, mortality was higher in patients with gram negative organisms or fungal sepsis [9].

117.2 Early Recognition and Treatment/ Resuscitation Improves Outcome in Sepsis and Septic Shock

Clinical signs of apnea or tachypnea, poor feeding and temperature instability should be considered as sepsis until proven otherwise and the clinician should consider antibiotic therapy. In the nursery setting, fetal and neonatal tachycardia remain the most important early clinical predictors of sepsis and septic shock [10, 11]. Prompt fluid and inotrope resuscitation to normalization of heart rate well before hypotension occurs appears prudent in this population. Laboratory tests can also have a role in increasing early suspicion of newborn sepsis. Many investigators have demonstrated that measurement of cord or newborn blood quantitative levels of cytokines including interleukin-6 (IL-6), procalcitonin, IL-1 receptor antagonist, IL-8, IL-10, tumor necrosis factor (TNF), and C-reactive protein (CRP) can attain over 95% sensitivity in diagnosis of EOD and LOD before blood culture results are available [12–20]. These author's believe that universal implementation of these clinical laboratory tests will allow more judicious use of antibiotic therapy than the present standards of care, which do not utilize these biomarkers.

Bang and colleagues [21] evaluated the effect of early treatment of sepsis on neonatal outcome in rural India when a rural health intervention initiative taught health workers to give intramuscular (IM) gentamycin and oral co-trimoxazole to premature and term newborns who developed apnea,

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

tachypnea, poor feeding, temperature instability or diarrhea. Remarkably, fatality rates were decreased from 16.5 to 2.8%. The cost of treatment per infant was $ 5 US. Interestingly only 7% of newborns in this study met these criteria and received antibiotics. In developed countries, 10% of newborns similarly receive antibiotics either antepartum or postpartum.

Shock remains the most prominent risk factor for death in neonatal sepsis (adjusted odds ratio 11.82 confidence interval 5.4–69.4) [15]. Best outcomes are attained with early reversal of shock in newborns using NRP/ACCM/PALS guidelines (see below) [22]. Aggressive, timely emergency department fluid and inotrope resuscitation directed to oxygen delivery/consumption goals has also been shown to improve outcome in adult septic shock in a large randomized controlled trial [23]. This preponderance of evidence suggests that early recognition is the key to survival in newborn sepsis and septic shock. Experimental and clinical literature show that early and aggressive fluid resuscitation with antibiotic therapy turns off inflammatory gene expression, prevents thrombosis, and results in 95% or greater survival. Delayed resuscitation on the other hand results in inflammatory gene expression, endothelial activation, thrombosis, the development of thrombocytopenia associated multiple organ failure, and high mortality rates [24–27].

117.3 Neonatal Shock

Adult septic shock is classically described as a high cardiac output and low systemic vascular resistance state. The systemic vasodilatation reduces afterload on the heart, thereby improving cardiac output. Death is usually attributed to refractory vasoplegia. In response to the decreased cardiac function (decreased stroke volume) adults compensate in part by tachycardia (Table 117.1). In contrast, term newborns and infants have a remarkably different cardiovascular response to septic shock. Healthy newborns have higher resting heart rates than adults. Hence, in response to shock, newborns compensate by increasing systemic vasoconstriction. This increased vasoconstriction increases afterload and further impairs cardiac output. Hence, death in the majority of newborns and infants with fluid/dopamine resistant shock is a result of cardiac failure, not vasoplegia.

Table 117.1 Clinical definition of sepsis and septic shock

Sepsis	Clinical suspicion of infection with the following signs; tachycardia, tachypnea or apnea, poor feeding, temperature instability, diarrhea
Septic shock	Clinical suspicion of infection with the following signs; tachycardia or bradycardia, tachypnea or apnea,poor peripheral perfusion, prolonged capillary refill > 2 seconds, decreased urine output*

* Hypotension is considered a late confirmatory sign.

Neonatal septic shock is often complicated by lack of the physiologic transition from fetal to neonatal circulation. *In utero*, 85% of fetal circulation bypasses the lungs through the patent ductus arteriosus and foramen ovale. This flow pattern is maintained by suprasystemic pulmonary artery pressures prenatally. At birth, inhalation of oxygen triggers a cascade of biochemical events that ultimately result in reduction in pulmonary artery pressure and transition from fetal to neonatal circulation with blood flow now being directed through the pulmonary circulation. Closure of the patent ductus arteriosus and foramen ovale complete this transition. Pulmonary artery pressures can remain elevated and the ductus arteriosus can remain open for the first 6 weeks of life. Sepsis-induced acidosis and hypoxia increases pulmonary vascular resistance, subsequently increasing the pulmonary artery pressure leading to patent ductus arteriosus. This results in persistent pulmonary hypertension (PPHN) and persistent fetal circulation (PFC) in the newborn. Neonatal septic shock with PPHN is associated with increased right ventricle afterload. Despite in utero conditioning, the thickened right ventricle may fail in the presence of systemic pulmonary artery pressures. Decompensated right ventricle failure can be clinically manifested by tricuspid regurgitation and hepatomegaly. Newborn animal models of Group B streptococcal and endotoxin shock have also documented reduced cardiac output, and increased pulmonary, mesenteric, and systemic vascular resistance. Therapies directed at reversal of right ventricle failure, through reduction of pulmonary artery pressures, are commonly needed in neonates with fluid refractory shock and PPHN. New therapies such as inhaled nitric oxide, extracorporeal membrane oxygenation (ECMO), and vasodilators have little to no role in adult septic shock, but are potentially lifesaving in newborns, infants, and children.

117.4 Management of Neonatal Shock

Management of neonatal shock (Fig. 117.1) is challenging due to many factors. First, there is lack of consensus about the definition of hypotension in the extremely premature infants [28]. The commonly used NICU criteria for mean arterial blood pressure is that it should be maintained at or greater than the mean gestational age in weeks [29, 30]. This is not supported by any published evidence but continues to be the standard of care in many NICUs around the world. In the absence of a precise evidence based definition, it is difficult to interpret the hemodynamic response to hypotension. Adult and pediatric data suggest that blood pressure cannot be used as a surrogate for adequate oxygen delivery in critically ill patients. Secondly, there is lack of data about the correlation of central venous pressure (CVP) with circulating blood volume in VLBW infants. Lastly, there is paucity of data regarding the use of lactate as a surrogate for inadequate tissue oxygen delivery in preterm infants. Serum lactate levels pose a unique challenge

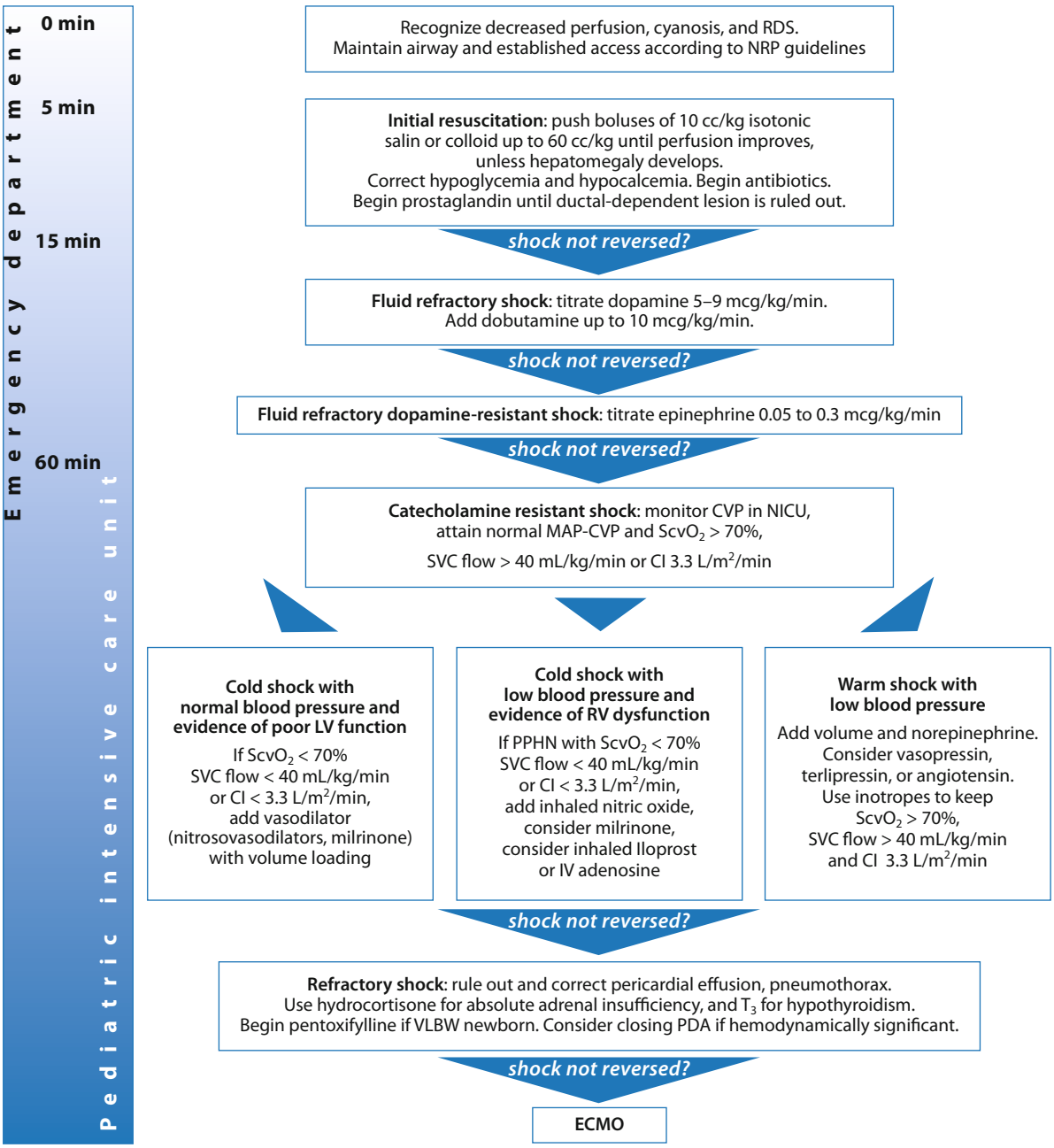

Fig. 117.1 Algorithm for time sensitive, goal-directed stepwise management of hemodynamic support in newborns. Proceed to next step if shock persists. 1) **First hour goals** Restore and maintain heart rate thresholds, capillary refill ≤2 sec, and normal blood pressure in the first hour. 2) **Subsequent intensive care unit goals** Restore normal perfusion pressure (*MAP-CVP*: mean arterial pressure-central venous pressure), preductal and postductal O_2 saturation difference <5%, and either central venous O_2 saturation ($ScvO_2$) >70%, superior vena cava (*SVC*) flow >40 mL/kg/min or cardiac index (*CI*) >3.3 L/min/m^2 in neonatal intensive care unit (*NICU*). *RDS* respiratory distress syndrome, *NRP* neonatal resuscitation program, *PDA* patent ductus arteriosus, *ECMO* extracorporeal membrane oxygenation

as neonates have high blood lactate concentrations at birth. These levels normalize by 12 hours after birth. Wardle and colleagues examined two groups of ventilated preterm infants with mean gestational age of 27 weeks. They found no difference in median lactate levels between normotensive and hypotensive preterm infants (1.20 *vs* 1.22 mmol/L). Despite the median lactate levels being similar in the two groups, persistent high lactate levels were associated with an adverse outcome (death or peri-ventricular hemorrhage) [31]. Thus, recognition and treatment of shock in low birth weight infants should encompass clinical signs (peripheral pulses, perfusion and urine output) along with biochemical values, i.e., serum

lactate levels. Standard practices in resuscitation of premature infants in septic shock employ a more graded approach compared to resuscitation of term neonates and children. This more cautious approach is a response to reports that premature infants at risk for intraventricular hemorrhage (< 30 weeks gestation) can develop hemorrhage after rapid shifts in blood pressure [32, 33]; however, some now question whether long-term neurologic outcomes are related to periventricular leukomalacia (a result of prolonged under perfusion) more than to intraventricular hemorrhage. To summarize, while cerebral under perfusion is a set up for periventricular leukomalacia, aggressive resuscitation of critically ill VLBW can predispose them to cerebral hemorrhages. Hence the resuscitation of a VLBW neonate is challenging and needs to be studied further.

Several other developmental considerations influence therapies for shock. Relative initial deficiencies in the thyroid and parathyroid hormone axes have been appreciated and can result in the need for supplementation with thyroid hormone, and/or calcium replacement. Adrenal insufficiency and the need for hydrocortisone has been documented in this population as well [34]. In a double-blind, randomized, controlled study, 48 VLBW infants who had refractory hypotension and required dopamine (> 10 μgm/kg/ min) were assigned to receive stress dose of hydrocortisone (1 mg/kg Q8H) for 5 days or placebo solution. A significantly higher number of hypotensive VLBW infants treated with hydrocortisone were weaned off vasopressor support 72 hours after starting treatment. There was decreased use of volume expanders and less cumulative dose of dopamine and dobutamine in steroid treated patients as compared to control infants [35]. Other factors that impact the neonate's response to shock include reduced glycogen stores and muscle mass. These are important for gluconeogenesis; hence attention should be paid to maintenance of serum glucose in a critically ill neonate.

Studies of therapies specifically directed at premature very low birth weight infants with septic shock are needed. A single center randomized control trial reported improved outcome with use of daily 6 hour pentoxifylline infusions (a systemic vasodilator) in very premature infants with sepsis. The Cochrane analysis agrees that the smaller trials are promising but suggests that larger multicenter trials would be helpful [24].

The American College of Critical Care Medicine (ACCM) set a priority to establish clinical practice parameters and guidelines for the management of septic shock. As a follow-up to their original guidelines published in 2002, ACCM issued an update published in 2007 [36, 37]. Although the intent was to develop guidelines for the management of premature as well as term newborns, the literature review on septic shock in the premature was relatively sparse. The evidence and expert opinion-based document found age-specific differences in both pathophysiology and response to therapies [38–74]. Rapid fluid resuscitation is the hallmark of therapy. It should be implemented when newborns are in compensated shock and directed to reversal of tachycardia. Newborns with fluid refractory shock can have any hemodynamic state. Some have the classic adult form of septic shock with high cardiac output and low vascular resistance; however, the majority have a low cardiac output state commonly associated with elevated, not reduced vascular resistance. If there is heightened concern for a ductal dependent heart lesion, all newborns with shock should be started on prostaglandin E. Newborns have an age-specific resistance to dopamine and dobutamine, hence epinephrine (cold shock) can be more commonly required. Newborns with septic shock have a higher incidence of true adrenal insufficiency (cortisol < 18 μg/dL) when requiring epinephrine. These patients may benefit from hydrocortisone therapy. In patients with low cardiac output and elevated systemic vascular resistance, vasodilators are effective in reversing shock. ECMO is life saving for refractory newborn septic shock. Inhaled nitric oxide decreases ECMO use but does not improve outcome in ECMO centers. ECMO is recommended for term infants with refractory shock.

117.5 Thrombocytopenia Associated Multiple Organ Failure

Thrombocytopenia, platelet count < 100,000 mm^3, is an independent risk factor for the development of multiple organ failure and death in critical illness, in part because it is a recognizable clinical sign of endotheliopathy with platelet thrombi. This syndrome is accounted for by variations on two prototype thrombotic microangiopathies. The consumptive coagulopathy (reduced fibrinogen levels) occurs when tissue factor complexes with factor VII and initiates the coagulation factor consumption cascade. This is commonly called disseminated intravascular coagulation (DIC). Román and colleagues have documented that newborns with sepsis/septic shock have a pro-thrombotic/anti-fibrinolytic state with excessive thrombosis – pro-coagulant (Factor II, VII) and anti-coagulant factors (i.e., protein C and anti-thrombin III) are both consumed [26, 27]. This leads to a paradoxical observation, overwhelming thrombosis (when the anti-coagulant factors are depleted) and then overwhelming bleeding (when the pro-coagulant factors are consumed). Newborns have lower protein C levels than children and adults. Activated protein C (APC) has 40 times the fibrinolytic activity as protein C concentrate and it is associated with intracranial bleeding. The pediatric arm of the "Extended Evaluation of Recombinant Activated Protein C" or ENHANCE trial noted increased incidence of significant bleeding in 27% of the patients enrolled and 3% had a central nervous system bleed [75]. Due to the adverse risk to benefit ratio associated with use of APC, it is not being used by most practitioners.

The second prototype is a non-consumptive coagulopathy (normal or increased fibrinogen levels), which is characterized by low ADAM TS 13 activity leading to platelet thrombi. Newborns have lower ADAM TS 13 activity than children and adults. This condition has been named infection-associated

thrombotic thrombocytopenic purpura. It responds to daily centrifugation-based plasma exchange therapy for a median of 14 days. Plasma exchange machines are not approved for infants under 5 kg so their use is limited in the newborn period. Nevertheless, newborns have reduced ADAM TS 13 activity and increased circulating ultra large vWF multimers (thrombogenic multimers) which puts the newborn at risk for non-consumptive coagulopathy. The use of fresh frozen plasma remains a standard approach to both forms of coagulopathy in newborns. Some have reported improved outcome with whole blood exchange but not blood component exchange therapy [76, 77].

117.6 Why Do Newborns Have Difficulty Eradicating Infection?

117.6.1 Hypogammaglobulinemia

Newborns and premature newborns in particular have a reduced ability to eradicate infection at almost all levels of immunity. Most notorious is the common deficiency of IgG levels in the VLBW infant. Prophylaxis with intravenous immunoglobulin (IVIG) therapy has not reduced late onset sepsis in this group of patients, but IVIG therapy in newborns with hypogammaglobulinemia and septic shock is thought to be of benefit. A recent meta analysis demonstrated that the use of immunoglobulin preparation rich in IgG, IgA and IgM reduces the mortality in neonatal sepsis or septic shock by 50% [78]. Hence, IVIG therapy should be considered in newborns with hypogammaglobulinemic septic shock or toxic shock [79–82].

117.6.2 Neutropenia

Neutropenia is commonly seen in newborns with sepsis/septic shock. Some have defined it as an absolute neutrophil count < 1500/mm^3. Granulocyte macrophage-colony stimulating factor (GM-CSF) therapy at 5 μg/kg/day over 12 hours for 7 days has been reported to improve outcomes in newborns with neutropenic septic shock [83]. G-CSF has also been studied in newborns with non-neutropenic sepsis and was found to be associated with a shortened length of stay [84]. In a multicenter trial in the United Kingdom, 280 small for gestational age neonates of ≤ 31 weeks gestation were randomized within 72 h of birth to receive GM-CSF 10 μg/kg per day subcutaneously for 5 days or standard management. The primary outcome was sepsis-free survival to 14 days from trial entry. The investigators found that neutrophil counts increased significantly more rapidly in infants treated with GM-CSF than in control infants during the first 11 days, however there was no significant difference in sepsis-free survival for all infants [85]. Hence, GM-CSF can be used to increase the neutrophil count but may not provide any survival benefit in septic neonates [86–90].

117.6.3 Prolonged Monocyte Deactivation and Immune Paralysis

Monocyte deactivation (< 30% HLA-DR expression or 8,000 HLA-DR molecules, or ex vivo whole blood tumor necrosis factor [TNF] response < 200 pg/mL for > 5 days) is associated with immune paralysis and increased risk of late onset sepsis from a secondary infection in children and adults [91]. Hallwirth and collegues have reported that cord monocyte deactivation is a reliable parameter for predicting EOD in VLBW infants [92]. The anti-inflammatory cytokine, IL-10, and the reactive oxygen species, nitric oxide, and peroxynitrite radicals both deactivate monocytes. This common response becomes pathogenic when it lasts for more than 5 days. GM-CSF and interferon reverse this process *ex vivo*.

117.6.4 Prolonged Lymphopenia and Lymphoid Depletion Syndrome

Lymphopenia (< 1000/mm^3 for > 7 days) is associated with the development of secondary infection, unresolving multiple organ failure, and the finding of lymphoid depletion at autopsy in children [93]. Gurevitch et al [94] examined autopsies from low birth weight newborns with sepsis similarly reported lymphoid depletion. At present prophylactic and empiric antifungal/antiviral strategies may be appropriately considered in these patients as per the clinical experience with patients with low CD4 counts from other immunodeficiency diseases. IVIG therapy should be considered if B-cell numbers are substantially depleted along with hypogammaglobulinemia (IgG level < 500 mg/dL).

117.6.5 Antibiotic Prophylaxis, Empiric Therapy and Antibiotic Resistance

Because EOD is commonly caused by GBS and LOD is commonly caused by *Staphylococcus epidermidis*, antibiotic prophylaxis therapies have been considered (Table 117.2). Antepartum and intrapartum antibiotic use has markedly reduced the incidence of GBS EOD; but not surprisingly, has led to increased incidence of neonatal sepsis caused by resistant organisms. Vancomycin and teicoplanin prophylaxis are both effective in reducing LOD with *Staphylococcus* species [95], but routine prophylaxis is not yet recommended because of concern for emergence of resistant organisms. Fluconazole

Table 117.2 Suggested treatments to reverse immune insufficiency and/or to prevent nosocomial infection and sepsis

Sepsis/MODS	Neutropenia	Prolonged lymphopenia ALC < 1000/mm^3 for 7 days, or IgG < 500 mg/dL	Monocyte deactivation HLA-DR < 30% or 8,000 molecules > 5 days
Give GM-CSF	Yes		Yes?
Give IVIG		Yes if IgG < 500	
Consider empiric and prophylactic antibiotic/protozoal fungal strategies	Yes	Yes	Yes?

prophylaxis has been very effective in preventing *Candida* sepsis in VLBW infants and is recommended [96]. Empirical antifungal therapy should be strongly considered for infants with gestational age < 25 weeks, thrombocytopenia, history of third-generation cephalosporin or carbapenem exposure for 7 days [97]. Empiric use of amphotericin for VLBW babies with risk factors for fungal infection has also been recommended [98, 99].

117.7 Supporting Organs During Multiple Organ Failure: What's New?

117.7.1 Acute Respiratory Distress Syndrome

Overdistention of alveoli results in systemic inflammation with systemic release of inflammatory cytokines and depression of immunity. Lung protection ventilation strategies which limit volutrauma are prudent [100].

117.7.2 Acute Renal Failure

Investigators have demonstrated the efficacy of continuous veno-venous hemofiltration in children with meningococcal septic shock [101]. A recent randomized controlled adult study showed survival benefit with daily dialysis compared to intermittent dialysis therapy in patients with acute renal failure in the ICU [102]. Peritoneal dialysis/hemofiltration, continuous veno- venous hemofiltration, or continuous arterio- venous hemofiltration can be successfully performed in newborns [103].

117.7.3 Insulin Therapy

An adult randomized controlled trial showed a survival benefit in patients with sepsis/multiple organ dysfunction syndrome (MODS) when insulin was used to maintain normal glucose levels during critical illness [104].

117.7.4 Steroid and Drug Metabolism

Reactive oxygen species impair cytochrome P450 activity. This leads to reduction of cortisol and aldosterone synthesis and reduced drug metabolism during sepsis and multiple organ failure. Newborns have an age-specific reduced cortisol synthesis for a given substrate (17-OH progesterone), as well as reduced drug metabolism due to an immature cytochrome P450 system [105].

Relative adrenal insufficiency is considered pathologic in adults with catecholamine resistant septic shock and mortality is reduced when these patients are treated with a 7 day course of hydrocortisone and fludrocortisone [106]. Hydrocortisone has reversed epinephrine resistant septic shock in premature infants with adrenal insufficiency. In a retrospective observational study, 117 infants treated with hydrocortisone for refractory hypotension were reviewed. Refractory hypotension was defined as a mean arterial pressure (MAP) less than the gestational age (GA) despite a total inotrope dose of 20 μg/kg/min. Patients treated with hydrocortisone demonstrated improved hemodynamics and decreased inotropic dose at 6, 12 and 24 h. The incidence of side effects, i.e., intraventricular hemorrhage, periventricular leukomalacia, sepsis and spontaneous intestinal perforation was similar to institutional historic controls [107].

117.8 Summary

Newborn sepsis remains a major international health care problem, particularly in low birth weight infants. It is a significant cause of mortality and neurologic morbidity including cerebral palsy. As with all major health care problems, resources should be invested in prevention and early intervention programs. Antepartum GBS prophylaxis has been successful in reducing incidence and mortality.

When sepsis is not recognized and treated early, septic shock becomes the predominant predictor of mortality and neurologic morbidity. In contrast to adults, septic shock in neonates occurs primarily secondary to cardiac failure, not vascular failure. This state of cardiac failure is commonly

associated with systemic pulmonary hypertension. Hence therapies including volume resuscitation, inotropic support and right ventricle afterload reduction are the mainstay. ECMO is life-saving for term newborns with refractory shock. At present no specific therapies have been approved for thrombotic complications (DIC or TTP/HUS) in newborns, hence plasma therapies remain the mainstay.

Sepsis is all too common in very low birth-weight newborns (20–30% of the neonatal population) with a predominant late (after 72 hrs) rather than early onset. Development of early diagnostic tests, infection prevention practices, and immunostimulant therapies are urgently needed for this population not only to improve survival but to reduce periventricular leukomalacia.

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Neonatal Viral Infections: Enteroviruses, and Respiratory Syncytial Virus

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118.1 Enteroviruses

118.1.1 Introduction

The detection and identification of viral infections in newborns has been greatly improved by the recent development of rapid and highly sensitive diagnostic methods, particularly polymerase chain reaction (PCR) techniques. Data from a retrospective analysis of neonates admitted to a Neonatal Intensive Care Unit (NICU) in the Netherlands during a 12 year period confirmed viral infection in about 1% of cases. Enterovirus or parechovirus (EV/PeV) were the most frequent causes (39% of cases), followed by respiratory syncytial virus (RSV) at 29% (Table 118.1). EVs caused the highest mortality rate and serious sequelae [1].

EV and PeV are non-enveloped, single-stranded RNA viruses belonging to the Picornaviridae family (pico = small). Until 1999, EV were divided into five classes, based on their replication properties in tissue culture and animal models: polioviruses 1–3, Coxsackie viruses (Coxsackie virus A, Coxsackie virus B), echoviruses, and EV. On the basis of sequence analysis, the EV genus was recently reclassified into five species. Polioviruses and Human EV A, B, C, and D. Echovirus 22 and echovirus 23 were reclassified as PeV1 and PeV2 genuses, respectively. New PeVs have recently been identified: PeV3 and PeV4 [2].

Table 118.1 Viral infections in a Neonatal Intensive Care Unit over a 12-year period (number of infants 5396; viral infections 51)

Virus	Percentage
Enterovirus/Parechovirus	39%
Respiratory syncytial virus	29%
Rotavirus	10%
Cytomegalovirus	6%
Adenovirus	4%
Parainfluenza virus	4%
Herpes simplex virus	4%
Rhinovirus	2%
Rubella	2%

Data from [1].

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118.1.2 Epidemiology and Pathogenesis

EV and PeV infections occur throughout the year, but mostly during the summer and autumn in temperate climates [2]. EVs spread from person-to-person by fecal-oral or oral-oral (respiratory) routes. Transmission has been documented by the contaminated hands of health care personnel and by fomites. Mother-to-child transmission may occur in utero, during or after delivery. Disease severity is greater in neoantes than in older children. The pathogenesis and pathology of EV infections depend on virulence, tropism and inoculum virus concentration, as well as host factors.

The portal of entry is the pharynx and lower alimentary tract. Within one day, the infection extends to the regional lymph nodes. On about the third day, minor viremia occurs with subsequent spread to secondary infection sites {e.g., liver, heart, central nervous system [CNS]) where virus replication causes local damage. Within a week, there is major viremia with associated clinical manifestations. EVs are usually cytolytic. Symptoms and signs are related to the extent and location of cell death. Cessation of viremia correlates with the appearance of serum antibody. Infection may persist in the lower intestinal tract.

118.1.3 Clinical Aspects

In neonates, most nonpolio EV infections are asymptomatic although they can cause mild, nonspecific febrile illness,

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

occasionally with rash. Their onset is characterized by mild irritability, a fever varying between 38°C and 39°C, frequently accompanied by poor feeding and, in some infants, by vomiting and/or diarrhea [3].

A serious complication of nonpolio EVs is a sepsis-like illness, which has been most frequently observed with Coxsackie virus types B2 to B5 and echovirus types 5, 11 and 16. It is characterized by high fever, abdominal distension, irritability, lethargy, and hypotonia. Other clinical features include diarrhea, vomiting, seizures, shock, disseminated intravascular coagulation, thrombocytopenia, hepatomegaly and jaundice [4].

Respiratory illness associated with non-polio echoviruses is sporadic, except for echoviruses 11 and 22. The clinical presentation includes rhinitis and pharyngitis, and, to a lesser extent, laryngitis or interstitial pneumonitis. Coxsackie virus A9 and B4 or echovirus 9, 11, 17 and 31 are more frequently associated with pneumonia [4].

Coxsackie viruses and echoviruses can cause vomiting, diarrhea and hepatitis with various degrees of hepatic necrosis. Some Coxsackie virus serotypes can cause pancreatitis or necrotizing enterocolitis [4].

Most cases of neonatal myocarditis are caused by Coxsackie virus B infections and, less frequently, by echovirus 11 and 19 [4]. There is often an abrupt onset with listlessness, anorexia and fever. Progression is rapid and there may be circulatory failure (tachycardia, cardiomegaly, electrocardiographic changes and transitory systolic murmurs, respiratory distress and cyanosis). The mortality rate is high.

Neonatal exanthema has been noted with Coxsackie virus B1 and B5 and other echoviruses, a macular, maculopapular, or (rarely) petechial rash appearing between the third and fifth day of the illness [4].

Echoviruses, particularly Coxsackie virus B1 to B5, may cause meningitis and meningoencephalitis. Cerebrospinal fluid (CSF) examination shows variations in protein, glucose and cellular values, which may mirror a bacterial disease. In particular, hypoglycorrachia is noted in about 10% of newborns with enteroviral meningitis.

A monolateral facial paralysis with transitory loss of abdominal reflexes due to Coxsackie virus B2 has been described [4].

Coxsackie virus A4, A5, A8, B3, echovirus 11 and 22 have been related to sudden infant death syndrome.

Postnatally acquired poliovirus infection may be asymptomatic or cause various symptoms and signs, including paralysis (Table 118.2). Some infants who acquired poliomyelitis died within the first 2 weeks of life. Neonatal infections due to PeV1 or 2 are observed sporadically and mostly cause mild gastrointestinal and respiratory disturbances. Recently, the new PeV3 has been associated with severe disorders, including involvement of the CNS with mild-to-severe white matter injury. PeV4 was isolated from an infant with fever [2].

The diagnosis is by virus culture, direct antigen detection and/or PCR.

118.1.4 Differential Diagnosis

Hypothermia and hyperthermia in association with other nonspecific signs are common in neonatal enteroviral infections and in bacterial sepsis. An increase in serum C-reactive protein concentrations, considered indicative of bacterial infection, is also seen in infants with EV infections and less frequently in patients with PeV infections. In meningitis, the CSF findings in bacterial and viral illnesses may be similar and bacterial culture and PCR assays are essential. Skin lesions are common in generalized herpes simplex infections and scraping a lesion should allow a rapid diagnosis.

Neonatal seizures due to enteroviral meningoencephalitis may be difficult to differentiate from seizures related to hypoxic-ischemic encephalopathy in full-term infants. The differences are that patients with enteroviral infection may have a history of a viral infection in the family and an uneventful pregnancy and delivery, later onset of seizures, and a possible rash. Neonates with PeV infection cannot be distinguished

Table 118.2 Clinical manifestations of Enterovirus infection in newborns

General symptoms	Specific symptoms
No symptoms	–
Sepsis-like	Fever, poor feeding, abdominal distension, irritability, rash, lethargy and hypotonia, diarrhea, vomiting, seizures, shock, disseminated intravascular coagulation, thrombocytopenia, hepatomegaly, jaundice and apnea
Respiratory	Rhinitis, pharyngitis, laryngitis, herpangina, coryza, laryngotracheobronchitis, bronchitis, interstitial pneumonitis
Gastrointestinal	Vomiting and diarrhea, hepatitis, hepatic necrosis, pancreatitis, necrotizing enterocolitis
Cardiological	Tachycardia, cardiomegaly, electrocardiographic changes, transitory systolic murmurs, myocarditis, respiratory distress, cyanosis, signs of neurologic involvement
Dermatological	Macular or maculopapular or petechial rash
Neurological	Anorexia, fever, lethargy, jaundice, vomiting, seizures, apnea, tremulousness, general increased tonicity, meningitis, meningoencephalitis, paralysis
Sudden infant death	–

from those with EV infection by clinical signs only. There are similar white matter abnormalities on cranial imaging. However, the diagnosis may be made by reverse transcription PCR for EV and PeV on blood and/or CSF because of genetic differences between the viruses [2].

Fever or hypothermia, lethargy, weakness and characteristic electrocardiographic changes differentiate neonatal myocarditis from congenital heart disease.

118.1.5 Prognosis

Both EV and PeV may lead to severe cerebral disease with associated serious sequelae [2]. In one study of six infants with CNS involvement, three babies born preterm had abnormal neurodevelopmental outcomes, while the three born at term developed normally. This is likely to be due to the different vulnerability of the white matter during maturation [5].

Poliovirus infections in neonates are generally severe, causing death or persistent paralysis in a consistent number of patients.

118.1.6 Therapy and Treatments

There is no specific therapy for any EV infection. Human immune serum globulin may be considered when there is severe and generalized neonatal infection on the assumption that the infant did not receive protective antibodies from the mother.

Treatment is generally supportive. The use of corticosteroids in neonatal encephalitis, myocarditis or other severe illnesses, is debated with some authors taking a view that they should not be used during acute EV infections, with others supporting their use in Coxsackie virus myocarditis, although deleterious effects have been observed in mice.

Meningoencephalitis is often associated with convulsions, cerebral edema and fluid and electrolyte balance disturbances. Serum electrolyte levels and fluids should be closely monitored because inappropriate antidiuretic hormone secretion is common.

In case of serious outbreaks in a nursery, passive protection with immunoglobulins for all exposed infants should be considered. The worldwide use of vaccination with inactivated polio vaccine (IPV) and the oral polio vaccine (OPV) developed by Sabin in the early sixties made it possible to control the epidemic in most areas. However, logistic problems and the cost of vaccination have meant that poliomyelitis remains endemic in Central Africa and the Indian sub-continent [6]. Infants with paralytic poliomyelitis should be observed carefully for evidence of respiratory paralysis with positive-pressure ventilation for respiratory failure. Passive movements of the paralysed limbs should be started soon after the disappearance of fever.

118.2 Respiratory Syncytial Virus (RSV)

RSV is a ubiquitous paramyxovirus. It has a peak during the winter months. Neonates, particularly those born before 35 weeks of gestation, are at-risk of severe RSV infection and respiratory failure [7].

118.2.1 Epidemiology and Pathogenesis

RSV is extremely contagious. Transmission occurs by aerosol inhalation of droplet nuclei from an infected person. Contaminated objects may be important sources of infection and the hand carriage of contaminated secretions by nursery personnel may contribute to spread.

118.2.2 Clinical Aspects

RSV may be asymptomatic or cause a disease that varies from a mild afebrile upper respiratory tract infection to severe bronchiolitis or pneumonia with hyperinflated lungs and hypoxemia. The A subtype of RSV appears to be more serious than the B subtype. The risk of severe illness is highest in infants born prematurely and in those with underlying lung disease, significant congenital heart defects or immune deficiencies [7]. Infants younger than 1 month have higher viral titers in their secretions than older ones.

After a few days of incubation, the first symptom is usually non-purulent rhinorrhea, followed by cough and wheezing, with or without dyspnea, bronchiolitis, pneumonia and fever. Irritability, lethargy and poor feeding may be the initial manifestations in infants. Chest X-ray abnormalities may precede the clinical manifestations of lower respiratory tract involvement by a few days.

118.2.3 Diagnosis

Rapid diagnosis is made by detecting viral antigens in nasopharyngeal specimens by specific assays, such as immunofluorescent or enzyme immunoassay. Viral isolation may be difficult because RSV is a labile virus.

118.2.4 Prognosis

Many infants with severe RSV bronchiolitis experience recurrent wheezing in later childhood and there is evidence that early RSV bronchiolitis may predispose some infants to the development of childhood asthma, although this association remains poorly understood [7].

118.2.5 Therapy and Treatments

As RSV-related bronchiolitis and pneumonia may cause severe disease in high-risk infants, supportive care, such as intravenous hydration, management of bacterial complications and mechanical ventilation may be needed. The use of 3% saline and aerosolized ribavirin treatment for infants with lower respiratory tract infections caused by RSV remains debatable [8]. Candidates for ribavirin therapy are infants at risk for complications of RSV due to congenital heart disease, chronic lung disease or immunodeficiency, infants with severe illness and signs of respiratory failure or those with a prolonged illness because of an underlying medical condition [9].

Because of the limitations associated with the management of RSV disease, prevention remains paramount, especially in patients at high risk of severe disease. Palivizumab is a humanized mouse monoclonal antibody which has been shown to be a safe and efficacious, significantly reducing the burden of RSV-related disease in premature infants below 35 weeks of gestational age. Its use in such high-risk patients is recommended by specific national guidelines of a number of countries in Europe, America and Asia [10].

Palivizumab is administered at a dosage of 15 mg/kg in a single monthly intramuscular injection for 5 months during the epidemic season.

Important measures for the prevention and control of viral epidemics are careful hand washing by nursery personnel after handling each infant, restricting the nursery area to personnel and visitors free of respiratory illnesses, and barrier nursing sick infants.

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Vaccinations and Neonatal Immunity

Alberto G. Ugazio and Alberto E. Tozzi

119.1 Immunological Development of the Neonate

The fetus can be compared to a haploidentical graft, sharing only half of the histocompatibility antigens of the "mother host". As such, the fetus should be rejected by the immune system of the mother. At the same time, since the fetal immune system starts developing around the 9th week of gestation, the fetus should also mount a "graft-versus-host" reaction against those histocompatibility antigens that it has not inherited from the "mother host". It follows that survival of the fetus – indeed of all mammalian species – is strictly dependent on a wide variety of mechanisms preventing graft rejection by the mother and graft-versus-host reaction by the fetus [1]. Among the latter, a major role is certainly played by the slow intrauterine maturation of the fetal immune function, ultimately resulting in the physiological immune deficiency of the neonate that involves both innate and adaptive immunity. This immune immaturity – more severe in preterm and in very low birth weight infants - results in a weak immune response to antigen challenge and in the production of a poor immunological memory [2].

After birth, the immune responsiveness quickly matures in both term and preterm neonates, rapidly reaching levels comparable with those observed in children and adults. The duration of this maturation process is variable, and also depends on the type of antigen to which immunity is directed. The optimal period for vaccination is decided therefore on a balance between the need of protecting the newborn as soon as possible against potential infectious threats, and the opportunity to trigger protective and durable immune responses.

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119.2 General Recommendations

Routine immunizations are often delayed in preterm and low birth weight infants, due to their impaired ability to develop protective immunity, and to the perception that they are more susceptible to side effects [3–6]. However, the safety profile and immunogenicity of routinely administered vaccines are similar to those measured in full-term infants. Therefore, in most cases, preterm and low birth weight infants should be immunized according to their chronological age [7, 8]. In addition, vaccine dosage should not be reduced or divided [7].

The site of choice for intramuscular vaccine administration is the anterolateral thigh, and needle length should be chosen on the basis of the available muscle mass [8].

Preterm infants may suffer from cardiorespiratory instability at the time of immunization and vaccine administration has been suspected to trigger apnea, desaturation, and bradycardia, especially in children with pre-existing cardiorespiratory symptoms [9–15]. Moreover, infants that presented apnea in the 24 hours before the immunization have been considered more likely to develop post-immunization apnea [15]; post-immunization apnea has been reported to occur more frequently in the presence of severe illness at birth, younger age, and lower weight [15]. None of the cardiorespiratory or other adverse events observed in preterm or low birth weight infants had sequelae in the studies published so far, and all of them were transient [15, 16]. However, based on the data from these observational studies, it has been recommended that infants still hospitalized at the time immunization is due should be monitored in hospital for 48 hours after vaccine administration [16]. Severe cardiorespiratory events after the first vaccine dose may occasionally predict similar episodes at the second dose [17]. More recently, a randomized trial in preterm children immunized with DTaP demonstrated that the rate of prolonged episodes of apnea or bradycardia is similar in immunized infants and controls [18].

An increase in C reactive protein (CRP) is commonly found in infants after immunization, although such an increase

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has not been observed with HBV and IPV immunizations [14]. The finding of normal CRP values before immunization suggests that high CRP values following immunization are possibly induced by vaccine administration [14].

Early and timely immunization of preterm and low birth weight infants is safe and essential for protecting them against diseases to which these infants are particularly susceptible and that may even be fatal. The benefits of early and timely immunization of fragile children far exceed the risk of inducing suboptimal protection. In addition, immunization of households and contacts of preterm and low birthweight infants against pertussis and influenza may be effective in decreasing the likelihood of exposure before immunization (pertussis) and before full maturation of immune defence mechanisms (influenza).

119.3 Vaccines That Can Be Administered at Birth

Since the neonate is not able to mount robust immune responses to most vaccines, immunizations are mostly delayed until the second or third month of age. Nonetheless, in some circumstances where the risk of acquiring some infectious diseases is high, early administration of vaccines, soon or shortly after birth, is crucial for avoiding infections.

BCG BCG is administered to neonates in many countries where the prevalence of tuberculosis is high. Studies in which preterm babies were compared with term infants showed similar responses to the tuberculin reaction after immunization and comparable cellular immuno responses [19, 20]. In one study the authors found a weaker delayed hypersensitivity to tuberculin after BCG in preterm infants compared with full-term neonates, and therefore recommended to delay BCG administration in newborns younger than 33 weeks of gestational age [21]. Other authors found a positive relationship between a positive tuberculin test after BCG and postnatal weight [22]. Administration of BCG in preterm infants does not result in an increased reactogenicity compared with full-term babies [19].

Hepatitis B Virus (HBV) Immunization of newborns against hepatitis B together with hepatitis B immunoglobulin (HBIG) administration within 12 hours of birth is recommended in children born to hepatitis B surface antigen (HbsAg) positive mothers or to those whose HBV status is unknown [23]. It has been shown that HBV vaccine administration in newborns who weigh less than 2,000 g results in decreased seroconversion rates [24]. These children should be immunized as well within 12 hours of birth, although the initial vaccine dose should not be counted towards completion of the HBV series. In some countries as the US, HBV is administered to all newborns regardless of mother's HBV status [23]. Preterm infants weighing less than 2,000 g and born to HBsAg-negative mothers should receive the first dose of the HepB series at 1 month of chronological age or at hospital discharge in order to take advantage of the increased maturity of the newborn's immune system [25–27].

119.4 Vaccines That Can Be Administered in Infancy

As previously mentioned, immunization schedules start as a rule during the second to third month of life and usually include, in industrialized countries, diphtheria, tetanus, pertussis, polio, *Haemophilus* type b and hepatitis B vaccines. Some schedules also include either conjugated pneumococcal vaccine or conjugated meningococcal C vaccine. In developing countries the schedule may include a more limited number of vaccine components. Unfortunately, good quality efficacy and safety studies have been carried out only for a limited number of vaccines. A synthesis of the available evidence for each specific vaccine is presented below.

Diphtheria-Tetanus-Pertussis Vaccine Diphtheria, tetanus, and pertussis (DTP) immunogenicity has been demonstrated in different studies even when these components are combined with other vaccine antigens [28–31]. Conflicting results have been obtained for the immunogenicity of the pertussis component, with some studies showing low titres in preterm compared with full-term infants [28–31]. To date, a serological correlate of protection from pertussis has not been identified. Therefore, the finding of low titres of antibodies directed against antigenic components of the pertussis vaccine is of doubtful significance. Tolerability of vaccines including DTP antigens in preterm infants is similar to that observed in full-term babies [28, 29, 32].

Polio Vaccine Inactivated polio vaccine has been shown to be immunogenic and well tolerated in preterm infants even when administered within combined vaccines [29, 30, 33, 34]. Lower geometric mean titers have been observed for serotypes 2 and 3 in comparison with full-term infants [29, 35]. Oral polio vaccine, which is still in use in some countries, does not seem to be influenced in its response by gestational age [36].

Haemophilus Type b Vaccine (Hib) Tolerability of the Hib component has been shown in several studies carried out with combined vaccines [31, 32]. Percentage of responders and antibody titers have been shown to be lower in preterm infants [35, 37, 38]. Available data show an increase in vaccine failures in preterm compared with full-term infants when an accelerated schedule is used (2, 3, and 4 months of age) [39].

Hepatitis B Vaccine HBV is well tolerated in preterm and low birth weight infants and is fully immunogenic when administered in babies > 2,000 g or older than 60 days [29, 32, 40].

Conjugate Pneumococcal Vaccine Heptavalent conjugate pneumococcal vaccine seems to elicit a similar immune response in preterm and full-term infants [41, 42] although one study reported a lower immunogenicity in preterm infants immunized with an accelerated schedule (2, 3, and 4 months of age) [43]. Most importantly, efficacy of this vaccine in preterm and low birth weight infants is very high, similarly to what is observed in full-term infants [42]. Local reactions are observed more commonly in preterm and low birth weight than in full-term infants [42].

Conjugate Meningococcal C Vaccine The conjugate meningococcal C vaccine is well tolerated and immunogenic when administered to preterm infants, although the only available studies were performed with an accelerated schedule (2, 3, and 4 months of age) [44, 45].

Measles-Mumps-Rubella and Varicella A single study showed similar geometric mean titers and proportions of responders to measles, mumps, rubella and varicella in severely preterm as compared to full-term infants immunized at 15 months of age [46]. It must be taken into account that preterm infants lose maternal antibodies to measles early after birth [16]. Therefore, preterm infants may safely be immunized during outbreak periods between 6 and 9 months of age [16].

Rotavirus A pentavalent live attenuated rotavirus vaccine has proved safe and efficacious in preterm babies [47]. Although so far no epidemiological study has investigated the horizontal transmission of vaccine strains, vaccine virus shedding can be observed up to 15 days after administration. Children of appropriate age and still hospitalized may be immunized on the day of discharge.

Influenza One study carried out in infants born prematurely and immunized after 6 months of age with an inactivated influenza vaccine showed greater T-cell proliferative responses to influenza antigen in full-term children than in the preterm children, but similar proportions of responders [48].

119.5 Immunological Memory in Preterm Infants

A potential cause of concern is whether vaccines administered to preterm infants induce a good immunological memory. DTaP-IPV-Hib-HBV vaccine has been shown to induce immunological memory in most preterm infants primed with the same vaccine for all the components [38]. Other studies indicate that the immune response after primary immunization with conjugate meningococcal C or pneumococcal vaccine may be weaker in preterm than in full-term infants, and that it wanes after one year if an accelerated schedule is used (2, 3, and 4 months of age) [43, 45].

119.6 Children Treated with Corticosteroids

Preterm infants are often treated for variable periods with corticosteroids. Dexamethasone-treated preterm infants show significantly reduced antibody levels to diphtheria, tetanus, and to pertussis antigens after primary immunization [49]. It is not known how much this decreased response reflects on protection, especially for diphtheria and pertussis. A negative effect of dexamethasone treatment has been noted also on immune response to Haemophilus influenzae b vaccine [50], whereas it does not seem to decrease the immune response to conjugate meningococcal vaccine given at 12 months of age [51]. In general, care should be taken when dexamethasone-treated infants travel to endemic areas.

119.7 The Effect of Mercury Additives in Vaccines

Concern has been raised on the potential neurotoxicity of thimerosal containing vaccines administered early after birth. Since 1999, several international agencies including the European Medicine Agency and the Centers for Diseases Control have recommended that thimerosal be eliminated from vaccines as a precautionary measure [52, 53]. Epidemiological studies have shown that administration of thimerosal containing vaccines is not associated with an impaired neuropsychological development or with clear developmental disorders later in life [54–56].

119.8 The Future of Immunization of Newborns

Advances in immunization have been achieved throughout the last few decades bringing sophisticated means to protect children and the general population from severe transmissible diseases. Still, new approaches to the development of efficacious strategies to prevent some diseases are needed in the perinatal and infancy periods. Incidence of several diseases such as meningococcal and pneumococcal invasive infections, pertussis, and influenza is highest in infancy, and preterm babies often pay the highest toll in terms of morbidity. Moreover, given the relative immaturity of the immunologic system, these children, even when properly vaccinated, may not be optimally protected. Vaccines which stimulate the innate and the adaptive immune response may allow induction of better protection of the high risk newborn [57]. New routes of immunization may help to achieve the goal of inducing mucosal immunity [58].

Immunizing mothers to induce protection in the newborn may also represent a future development of immunization

strategies for protecting newborns [59]. Finally, new vaccines may help to prevent other diseases such as Group B streptococcal infections [60].

These goals may be achieved within a few years and provide us with new and more efficacious preventive tools for the special category of high risk newborns.

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Inborn Errors of Metabolism

Nicola Brunetti-Pierri, Giancarlo Parenti and Generoso Andria

120.1 Introduction

Inborn errors of metabolism are disorders of great importance to physicians treating newborns because rapid diagnosis and appropriate treatment of these conditions are directly related to the patient's outcome in terms of mortality and morbidity. Although individually rare, as a group these diseases are relatively frequent and collectively their incidence may approach 1 in 800 to 2500 births [1, 2]. The presentation of these disorders may occur in any age group, from fetuses and newborns to adulthood. Neonatal onset is common because the newborn period is a time of substantial catabolism. The main problems facing the physician caring for the sick newborn are when to consider an inborn error of metabolism, what test to order to determine quickly and efficiently whether the patient has an inborn error of metabolism, and what therapy to initiate given a specific or a suspected diagnosis. Unfortunately, given the limited repertoire of symptoms of the newborn, the early presentation is generally non-specific and usually includes poor feeding, breathing difficulties, lethargy, hypotonia, vomiting, hypothermia, and seizures. Therefore, patients with acute metabolic presentation are often misdiagnosed with other more common conditions such as sepsis, pulmonary disease, pyloric stenosis, and Reye syndrome. However, clues from the history, from the clinical presentation or from basic biochemical studies should raise the suspicion of a metabolic disease (Tables 120.1–120.7). A wide range of tests are required for the diagnosis of inborn errors of metabolism and the level of clinical and biochemical experience required is often substantial. Nevertheless, the neonatologist can initiate appropriate investigation with a relatively small number of laboratory tests which are readily available in most hospitals (Table 120.8). In many circumstances, the prevention of death or permanent neurologic sequelae is dependent on early diagnosis and institution of appropriate treatments. In addition, an accurate diagnosis is of primary importance for parental counselling.

Intensive efforts are currently ongoing to implement programs of expanded newborn screening using tandem mass spectrometry (MS/MS) with the goal of detecting a large number of inborn errors of metabolism in their pre-symptomatic early phase and have the potential to significantly change the natural history, the management, and the treatment of these disorders in the next few years.

120.2 Etiology and Pathogenesis

Metabolism is regarded as the sum of all chemical reactions that operate in a living cell. Enzymes play an essential role in facilitating each of these reactions serving as catalysts in the conversion of one metabolite to another. Inborn errors of metabolism are genetic disorders caused by alterations of specific chemical reactions in metabolism. The consequences of these alterations can be mediated by: 1) direct toxicity of accumulating upstream metabolites; 2) deficiency of downstream metabolites; 3) feedback inhibition/activation by the metabolite on the same or different pathway; and 4) diversion of metabolic

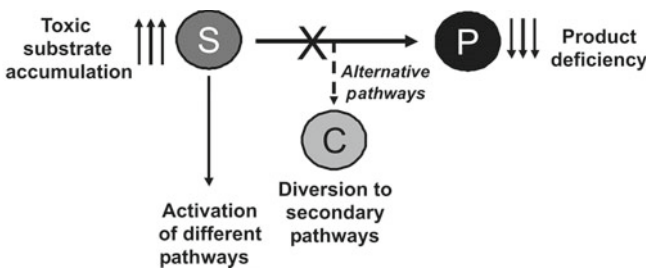

Fig. 120.1 An enzymatic blockage results in: 1) direct toxicity of accumulating upstream substrate (S); 2) deficiency of downstream product (P); 3) activation of alternative pathways; 4) diversion of metabolic flux to secondary pathways and alternative metabolite (C) production

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Table 120.1 Clues to the diagnosis of inborn errors of metabolism

- History
  - Consanguineity
  - Previous neonatal death
  - Recurrent nonimmune hydrops
  - Siblings with known inborn error of metabolism
- Clinical
  - Unexplained clinical deterioration in infant who was well at birth
  - Encephalopathy/coma/seizures
  - Persisting vomiting/feeding difficulties
  - Major organ failure (heart, liver)
  - Dysmorphism, multiple congenital anomalies
  - Unusual odors
  - Cataract
  - Bone marrow suppression
- Biochemical
  - Unexplained metabolic acidemia
  - Unexplained hypoglycemia
  - Ketosis
  - Hyperammonemia
  - Lactic acidemia
- Post mortem
  - Fatty liver or cardiomyopathy

flux to secondary pathways and buildup of toxic metabolites (Fig. 120.1). The majority of the inborn errors of metabolism are autosomal recessive but a few are X-linked. In addition, several of these disorders are included in the group of mitochondrial cytopathies which can exhibit matrilinear, autosomal recessive, autosomal dominant, and X-linked inheritance.

120.3 Clinical Presentations

The clinical presentation of metabolic diseases may include findings in virtually every system. From a clinical standpoint, inborn errors of metabolism can be classified according to the time of onset in prenatal or postnatal onset diseases. It is also useful to separate maternal inborn errors of metabolism affecting the fetus as well as fetal diseases affecting the mother. The postnatal group can be further classified according to the main symptom and laboratory findings at the time of disease onset [3]. It should be noted that patients with inborn errors of metabolism have often complex and overlapping problems which are a serious limitation of any classification system including the one used in this chapter.

Table 120.2 Inborn errors of metabolism with cardiac presentation in the neonatal period

	Main clinical features	Laboratory abnormalities	Diagnostic test
Fatty acid oxidation disorders	Cardiomyopathy Arrhythmias Hypotonia Sudden death	Hypoketotic hypoglycemia Hyperammonemia Elevated creatine kinase	Acylcarnitine profile Molecular analysis
Propionic acidemia and methylmalonic acidemia	Cardiomyopathy Encephalopathy Vomiting	Metabolic acidosis Bone marrow suppression	Organic acid analysis Acylcarnitine profile
Methylmalonic aciduria and homocystinuria CblC type	Structural cardiac defects	Metabolic acidosis Methylmalonic aciduria Increased blood homocysteine	Organic acid analysis Homocysteine determination
Tyrosinemia type I	Liver disease Cardiomyopathy	Elevated tyrosine	Elevated succinylacetone
Sugar alchol disorders	Progressive myocardial hypertrophy	Hypoalbuminemia; decreased coagulation factors	Urinary polyols
Mitochondrial disease	Cardiomyopathy Arrhythmias Hypotonia	Lactic acidosis Pancytopenia	Electron transport chain assay/ DNA testing
Pompe disease	Cardiomyopathy Hypotonia Wolf-Parkinson-White syndrome	Vacuolated lymphocytes Enlarged QRS complexes	Acid α-glucosidase activity
Glycogen storage disease type IV	Cardiomyopathy	Elevated creatine kinase	Glycogen branching enzyme activity
Heart-specific phosphorylase kinase deficiency	Cardiac enlargement	Hypoglycemia Short PR interval	Phosphorylase kinase activity
Mucopolysaccharidosis VI (Maroteux-Lamy disease)	Endocardial fibroelastosis Dysostosis multiplex	Increased urinary mucopolysaccharides	Galactosamine-4-sulphatase activity
Congenital disorders of glycosylation	Cardiomyopathy Pericardial effusion Hypotonia Dysmorphic features	Hypoalbuminemia Decreased coagulation factors Low thyroxine-binding globulin Increased transaminases	Transferrin isoelectrofocusing

Table 120.3 Inborn errors of metabolism with neonatal liver involvement

- Liver failure
  - Galactosemia
  - Tyrosinemia type I
  - α1-antitrypsin deficiency
  - Transaldolase deficiency
  - Mitochondrial DNA depletion
- Cholestasis
  - α1-antitrypsin deficiency
  - Galactosemia
  - Citrin deficiency
  - LCHAD deficiency
  - Cystic fibrosis
  - Niemann-Pick type C
  - Gaucher disease
  - Wolman disease
  - Peroxisomal disorders
  - Congenital disorders of glycosylation
  - Mitochondrial DNA depletion
  - Cholesterol biosynthetic defects
  - Bile acid synthetic disorders

Table 120.4 Hematological findings in inborn errors of metabolism

- Neutropenia
  - Organic acidemias
  - Barth syndrome
  - Lysinuric protein intolerance
  - Glycogen storage disease type Ib
- Neutropenia and pancytopenia
  - Organic acidemias
- Megaloblastic anemia
  - Cobalamin diseases
  - Transcobalamin II deficiency
  - Hypoxanthine-guanine-phosphoribosyl-transferase (HPRT) deficiency
- Pancytopenia
  - Mevalonic aciduria
  - Pearson syndrome
  - Tyrosinemia type I
  - Gaucher disease
- Acanthocytosis
  - Abetaliproteinemia
  - Wolman disease
- Vacuolated lymphocytes
  - Lysosomal storage disorders

Table 120.5 Skin and hair findings in inborn errors of metabolism

- Thick skin
  - I-cell disease
  - GM_1 gangliosidosis
  - Sialidosis
  - Galactosialidosis
  - Mucopolysaccharidosis VII
- Extensive Mongolian spots
  - Lysosomal storage diseases
- Hypopigmentation
  - Phenylketonuria
  - Cystinosis
  - Homocystinuria
- Hyperpigmentation
  - Glycerol kinase deficiency
  - Adrenoleukodystrophy
- Skin nodules
  - Farber disease (ceramidase deficiency)
  - Congenital disorders of glycosylation
- Desquamating eczematous or vesicobullous lesions
  - Multiple carboxylase deficiency
  - Methylmalonic acidemia
  - Propionic acidemia
  - Phenylketonuria
  - Glutamine synthetase deficiency
- Ichthyosis
  - Gaucher disease type II
  - Sjögren-Larsson syndrome
  - Multiple sulfatase deficiency
  - Multiple carboxylase deficiency
- Alopecia
  - Multiple carboxylase deficiency
- Kinky hair
  - Menkes disease
- Trichorrhexis nodosa
  - ASL deficiency

Table 120.6 Eye findings in inborn errors of metabolism

- Corneal clouding
  - Mucopolysaccharidosis
  - I-cell disease
  - Peroxisomal disorders
- Cataract
  - Galactosemia
  - Lowe syndrome
  - Peroxisomal disorders
  - Mevalonic aciduria
  - Mitochondrial diseases
  - Congenital CPT II deficiency
- Dislocated lens
  - Homocystinuria
  - Sulfite oxidase deficiency
- Macular cherry red spot
  - GM_1 gangliosidosis
  - Sialidosis
  - Galactosialidosis
  - Niemann-Pick type A
  - Tay-Sachs disease
- Retinopathy
  - Mitochondrial diseases
  - Methylmalonic acidemia
  - Sjögren-Larsson syndrome
  - Peroxisomal disorders
  - Abetalipoproteinemia
  - LCHAD deficiency
- Optic atrophy
  - Pyuvate dehydrogenase deficiency
  - Leigh disease
  - Peroxisomal disorders

Table 120.7 Clinical findings which are characteristic or unique in newborns with inborn errors of metabolism

- Microcephaly
  - L-serine biosynthesis disorders
  - Congenital CPT II deficiency
  - Smith-Lemli-Opitz syndrome
  - Mitochondrial diseases
  - Maternal phenylketonuria embriopathy
- Macrocephaly
  - Glutaric aciduria type I
  - Canavan disease
  - Lysosomal storage disorders
- Seizures
  - Biotinidase deficiency
  - Pyridoxine-dependent epilepsy
  - Pyridox(am)ine phosphate oxidase (PNPO) deficiency
  - Nonketotic hyperglycinemia
  - Methylene tetrahydrofolate reductase (MTHFR) deficiency
  - GABA transaminase deficiency
  - L-serine biosynthesis disorders
  - Urea cycle disorders
  - Molybdenum cofactor deficiency
  - Sulfite oxidase deficiency
  - Peroxisomal disorders
  - Congenital disorders of glycosylation
- Coarse facial features
  - Lysosomal storage disorders
- Macroglossia
  - Pompe disease
  - GM_1 gangliosidosis
- Skeletal abnormalities
  - Dysostosis multiplex
    - Lysosomal storage disorders
  - Chondrodysplasia punctata
    - Peroxisomal disorders
    - X-linked chondrodysplasia punctata (ARSE deficiency)
    - Conradi-Hunermann syndrome
    - Smith-Lemli-Opitz syndrome
    - GM_1 gangliosidosis
    - Galactosialidosis
    - Combined deficiency of multiple coagulation factors
  - Rhizomelia
    - Peroxisomal disorders (rhizomelic chondrodysplasia punctata)
  - Spondyloenchondromatosis
    - L-2-hydroxyglutaric aciduria
- Connective tissue abnormalities (cutis laxa, bladder divericula)
  - Menkes disease
- Persistent diarrhea
  - Congenital chloride diarrhea
  - Congenital lactase deficiency
  - Glucose/galactose malabsorption
  - Congenital sucrose-isomaltase deficiency
  - Galactosemia
  - Wolman disease
- Adrenal calcifications
  - Wolman disease
- Acure renal failure
  - HPRT deficiency
- Renal Fanconi syndrome
  - Tyrosinemia type I
  - Mitochondrial diseases
  - Congenital disorders of glycosylation
  - Cystinosis
  - Lowe syndrome
  - Galactosemia
  - Glycogen storage disease type I
- Renal cysts
  - Peroxisomal disorders
  - CPT II deficiency
  - Glutaric aciduria type II
  - Congenital disorders of glycosylation
- Increased LDH
  - Lysinuric protein intolerance
- Increased tryglycerides
  - Glycogen storage disease type I
  - Glycerol kinase deficiency (pseudo-increase)
  - Lipoprotein lipase deficiency
- Increased uric acid
  - MCAD deficiency
  - Glycogen storage disease type I
  - HPRT deficiency
- Decreased uric acid
  - Molybdenum cofactor deficiency
- Increased α-fetoprotein
  - Tyrosinemia type I
  - Ataxia-teleangectasia

120.4 Inborn Errors of Metabolism with Prenatal Onset

The fetus with a metabolic disorder is usually protected in utero by the metabolically normal mother through placental exchange of metabolites. Therefore, the majority of inborn errors of metabolism become clinically evident only after birth. The prenatal onset involves a group of disorders in which the maternal-placental unit cannot compensate for the underlying pathogenetic mechanisms thus resulting in fetal developmental defects. On a molecular basis, these disorders can be broadly divided into disorders involving large molecules and those of small molecules. Small molecule diseases involve organic acids, amino acids, carbohydrates, fatty acids, nucleotides, and ammonia. Large molecule diseases arise from either synthetic or degradative processing of polymeric molecules, such as glycoproteins and glycolipids.

120.4.1 Small Molecule Diseases

Small molecule diseases with prenatal onset present at birth with complex malformations rather than with acute decompensation. However, there is no rigid distinction and disorders

Table 120.8 Investigation for suspected inborn errors of metabolism

First line investigation
• Blood
– Complete blood counts
– Electrolytes
– Bilirubin
– Glucose
– Urea
– Blood gases and acid-base analysis
– Ammonia
– Lactate
– Uric acid
– Ketones
• Urine
– Reducing substances
– pH
– Ketones
• Cerebrospinal fluid
– Glucose
– Lactate
*Second line investigation**
• Urinary organic acid analysis
• Plasma, cerebrospinal fluid, urine amino acid analysis
• Plasma acylcarnitine profile
• Urine orotic acid
• Copper and ceruloplasmin
*Specialized investigation**
• Special biochemical assay (very long chain fatty acids [VLCFA], bile acid analysis, transferrin isoelectrofocusing, urine purine and pyrimidine, urine sugar alcohols)
• Enzyme assays
• DNA studies

* Before sending samples for second line for specialized investigations it is recommended to: 1) call the laboratory to indicate urgency, 2) give details on drug, diet, and previous blood transfusions, 3) arrange suitable transport of the samples, 4) discuss which tests are indicated with the metabolic consultants, and 5) freeze and save urine of the newborn.

such as nonketotic hyperglycinemia, which are associated with acute illness after birth, may also present with brain malformations. Similarly, mitochondrial diseases and fatty acid oxidation defects can exhibit features indicating prenatal onset damage [4, 5]. Accumulation of methylmalonic acid and homocysteine also appears to be involved in various types of congenital heart defects (Table 120.2) [6].

Disorders of serine biosynthesis are notable for their severe effects on prenatal development and present at birth with microcephaly, intractable seizures, and spastic tetraparesis. They are diagnosed by amino acid analysis in blood and cerebrospinal fluid (CSF) showing reduced levels of serine and glycine. The biochemical abnormalities are more pronounced in CSF rather than in plasma, and therefore, CSF amino acid analysis is preferable for the diagnosis. Early recognition of these defects is important because treatment with supplemental serine and glycine has beneficial effects [7].

Disorders of cholesterol biosynthesis have also significant effects on the fetus as exemplified by Smith-Lemli-Opitz syndrome, a multiple congenital anomaly syndrome caused by the deficiency of 7-dehydrocholesterol reductase catalyzing the reduction of 7-dehydrocholesterol to cholesterol in the last step of cholesterol biosynthesis. The clinical presentation of Smith-Lemli-Opitz syndrome is heterogeneous and severely affected infants have multiple major congenital anomalies of brain (holoprosencephaly, agenesis of the corpus callosum), heart (atrial and ventricular septal defects, atrioventricular canal defect), lungs (abnormal segmentation) or gastrointestinal tract (pyloric stenosis and colonic aganglionosis). Postnatal growth failure, microcephaly, typical facial features, ambiguous genitalia, limb anomalies such as postaxial polydactyly and syndactyly of the second and third toes are common findings. Desmosterolosis, lathosterolosis, X-linked chondrodysplasia punctata type II (Conradi-Hunermann syndrome), congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD syndrome), hydrops-ectopic calcification-moth-eaten skeletal dysplasia (HEM dysplasia), and a subset of cases with Antley-Bixler syndrome are due to defects in other steps of cholesterol biosynthesis and their clinical presentation partially overlap with Smith-Lemli-Opitz syndrome. These disorders are diagnosed by analysis of plasma sterols by gas chromatography/mass spectrometry (GC/MS) [8].

Another example of an inborn error of metabolism resulting in a complex malformative syndrome is the deficiency of glutamine synthetase presenting with severe brain malformations, multiorgan failure, and neonatal death [9]. The identification of this disorder on the basis of reduced glutamine levels is unusual because the interpretation of plasma amino acid profiles and the expanded newborn screening are usually directed toward increased (Table 120.9), rather than decreased metabolite levels.

120.4.2 Large Molecule Diseases

120.4.2.1 Lysosomal Storage Disorders

Lysosomal storage disorders are due to defects in lysosomal enzymes, cofactors, and transport proteins resulting in progressive accumulation of undegraded products within the lysosomes. These disorders usually present in infancy or early childhood with hepatosplenomegaly, skeletal dysostosis, and mental retardation. In the rare neonatal presentation they manifest as non-immune hydrops [10, 11]. The presence of dysmorphism, gum hyperplasia, inguinal and umbilical herniae, extensive Mongolian spots, and corneal clouding (Tables 120.5–120.7) [12] on physical exam and of placental vacuolization, vacuolated mononuclear cells in the peripheral smear, and bone marrow storage histiocytes on pathology are suggestive of these disorders [13].

A definitive diagnosis requires enzyme analysis which can be performed either on leukocytes or fibroblasts depending on the specific disease. Although lysosomal storage disorders represent the major cause, there is a growing awareness that several small molecules disorders can also result in

Table 120.9 Interpretation of plasma amino acid profile

Amino acid	Increase	Decrease
Alanine	Energy production defects Hyperammonemia	Maple syrup urine disease (during acute decompensation)
Alloisoleucine	Maple syrup urine disease	Normally not detected
Arginine	Argininemia Citrin deficiency	Prematurity Urea cycle disorders (deficiency of CPS, OTC, ASS, ASL)
Argininosuccinic acid	Argininosuccinic aciduria	Normally not detected
Citrulline	Citrullinemia Argininsuccinic aciduria Citrin deficiency Pyruvate carboxylase deficiency Lysinuric protein intolerance	OTC deficiency CPS deficiency
Glutamic acid	Sample mishandling	–
Glutamine	Hyperammonemia	Glutamine synthetase deficiency
Glycine	Nonketotic hyperglycinemia Organic acidemias	Disorders of serine biosynthesis
Histidine	Histidase deficiency*	–
Free homocysteine**	Homocystinuria† Methylenetetrahydrofolate reductase (MTHFR) deficiency	Isolated sulfite oxidase deficiency Molybdenum cofactor deficiency
Branched chain amino acids (Isoleucine, valine, and leucine)	Nonfasting Maple syrup urine disease	Hepatic dysfunction Ammonia scavenger treatment
Lysine	Hyperlysinemia*	Lysinuric protein intolerance
Methionine	Homocystinuria Methionine adenosyltransferase deficiency* Glycine-N-methyltransferase* Citrin deficiency Hepatic dysfunction	MTHFR deficiency
Ornithine	Ornithine aminotransferase I deficiency Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome	Lysinuric protein intolerance
Phosphoethanolamine††	Alkaline phosphatase deficiency	–
Phenylalanine	Phenylketonuria Hepatic dysfunction	–
Proline	Hyperprolinemia type I Hyperprolinemia type II	–
Serine	Nonfasting	Disorders of serine biosynthesis
S-sulfocysteine†††	Isolated sulfite oxidase deficiency Molybdenum cofactor deficiency	–
Threonine	Citrin deficiency Cholestasis Nonfasting	–
Tyrosine	Tyrosinemia type I Tyrosinemia type II Tyrosinemia type III Transient tyrosinemia of the newborn Citrin deficiency Hepatic dysfunction	–

* Clinical relevance of these biochemical abnormalities is not conclusive.
** Free homocysteine is usually not detected in plasma when standard amino acid analysis is performed.
† Can be present in cystathionine β-synthase or defects of vitamin B12 synthesis (*cblC*, *cblE*, *cblD*, *cblF*, *cblG*), absorption (hereditary folate malabsorption), and transport (transcobalamin II deficiency).
†† Phosphoethanolamine is not an amino acid but it is usually detected in the routine plasma amino acid analysis.
††† Measurement of sulfocysteine requires special sample preparation.

Table 120.10 Inherited metabolic diseases presenting as non-immune hydrops

Disease	Metabolic abnormality
Erythrocyte enzymopathies	
Glucose-6-phosphate dehydrogenase deficiency [51]	Glucose-6-phosphate dehydrogenase
Pyruvate kinase deficiency [51]	Pyruvate kinase
Glucose-phosphate isomerase deficiency [51]	Glucose-phosphate isomerase
Lysosomal storage diseases	
• Oligosaccharidoses	
– GM_1 gangliosidosis [52]	β-galactosidase
– Sialidosis [53]	Neuraminidase
– Galactosialidosis [52]	Protective protein/cathepsin A
– Gaucher disease type II [54]	Acid β-glucosidase
– Niemann-Pick disease, type A [52, 55]	Sphingomyelinase
– Niemann-Pick disease, type C [55]	NPC1
– Farber lipogranulomatosis [52]	Acid ceramidase
– Wolman disease [55]	Lysosomal acid lipase
– I-cell disease [52]	Multiple defects affecting mannose-6-phosphate receptor
– Infantile sialic storage disease [56]	Sialin
– Multiple sulfatase deficiency [57]	Sulfatase-modifying factor-1
• Mucopolysaccharidoses	
– Hurler disease (MPS I) [55]	α-L-iduronidase
– Sly disease (MPS VII) [56]	β-glucuronidase
– Morquio A disease (MPS IVA) [58]	Galactosamine-6-sulfate sulfatase
Others	
Congenital disorders of glycosylation type Ia; Ik; I/IIx [59–62]	Phosphomannomutase; β-1,4-mannosyltransferase; ?
Glycogen storage disease type IV [63]	Glycogen branching enzyme
Long-chain hydroxyacyl CoA dehydrogenase deficiency [64]	Long-chain hydroxyacyl-CoA dehydrogenase
Smith-Lemli-Opitz syndrome [65]	Sterol-Δ^7-reductase
3β-hydroxysterol-Δ^{14}-reductase deficiency [66]	3β-hydroxysterol-Δ^{14}-reductase
Citric acid cycle-defect [67]	Fumarase
Pearson syndrome and other respiratory chain defects [68]	Respiratory chain defects
Transaldolase deficiency [69]	Transaldolase
Congenital erythropoietic porphyria [70]	Uroporphyrinogen III cosynthetase

non-immune hydrops (Table 120.10). Besides hydrops, Gaucher disease may have a perinatal lethal presentation with colloidon membrane, hepatosplenomegaly, and arhtrogryposis [14]. In addition, a significant number of infants with Niemann-Pick type C presents in the newborn period with hypotonia, jaundice, hepatosplenomegaly, and liver failure [15]. Cardiomyopathy in the neonatal period has been reported as rare complication of glycogen storage disease type II (pompe disease) and of some muchopolysaccharidoses (Table 120.2) [12].

120.4.2.2 Peroxisomal Disorders

In contrast to lysosomal storage disorders, most peroxisomal disorders are expressed in the neonatal period. Zellweger syndrome, the prototype of these disorders, classically presents in the newborn period with craniofacial anomalies including a large anterior fontanel with widely spaced sutures, broad forehead and micrognathia, and neurological abnormalities. The presence of hypotonia and the hypo/areflexia in these patients may raise the possibility of spinal muscular atrophy [16]. Neuronal migration defects, especially pachygyria and polymicrogyria, are observed along with white matter abnormality and are often associated with seizures. The liver is enlarged and fibrotic with micronodular cirrhosis. Renal cysts and chondrodysplasia punctata are also common. Apart from Zellweger syndrome with its neonatal onset and early fatal course, the peroxisomal disorders include neonatal adrenoleukodystrophy and infantile Refsum disease with the least severe course. The clinical features between Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease are overlapping and it is often difficult to assign a particular patient to one of these clinical subtypes. Most peroxisomal defects can be detected by analysis of very long-chain fatty acids (VLCFA) in plasma. Additional studies such as plasmalogen levels in red blood cells, plasmatic phytanic acid, and molecular analysis are necessary for further confirmation of the diagnosis [17].

120.4.2.3 Congenital Disorders of Glycosylation

The congenital disorders of glycosylation are multisystemic diseases due to a deficiency of carbohydrate residues of glycoproteins and other glycoconjugates [18]. In the neonatal

period the neurologic picture comprises abnormal slow-rolling vertical or horizontal eye movements, axial hypotonia, and hyporeflexia. Many patients have dysmorphic features, abnormal adipose tissue distribution, inverted nipples, moderate hepatomegaly, and cerebellum hypoplasia. Some patients develop pericardial effusion and cardiomyopahty (Table 120.2) or non-immune hydrops (Table 120.10). The screening and diagnosis are based on plasma glycosylated transferrin by isoelectrofocusing.

120.5 Maternal Metabolic Diseases Affecting the Fetus

Phenylketonuria is a disorder with increased concentration of phenylalanine in the body fluids resulting from the reduced activity of the phenylalanine hydroxylase or from defects in the synthesis or recycling of its cofactor, tetrahydrobiopterin. Untreated patients with classic phenylketonuria are normal at birth, but develop microcephaly, seizures, and severe mental retardation. In contrast, affected children detected and effectively treated before the 3 weeks of age show none of these abnormalities. Therefore, most newborns in developed countries are screened by determination of phenylalanine blood levels. Women with phenylketonuria who have been treated since infancy and become pregnant have an increased risk for fetal malformations such as microcephaly, mental deficiency, and congenital heart disease [19]. These adverse consequences originate in the first trimester and are preventable by a phenylalanine restricted diet. There is a strong relationship between increasing phenylalanine levels and the severity of neonatal abnormalities. The current recommendation is to maintain the phenylalanine levels < 6 mg/dL at least 3 months before conception [20]. Maternal riboflavin and vitamin B12 deficiency may also affect the newborn. Maternal riboflavin deficiency results in clinical and biochemical findings of multiple acyl-CoA dehydrogenase deficiency [21], while maternal vitamin B12 deficiency, often secondary to vegetarian diet, pernicious anemia, and gastric bypass, results in non-specific clinical presentation with developmental delay, failure to thrive, and irreversible neurologic damage. Early detection and intervention, facilated by MS/MS newborn screening [22], are critical and both conditions can be treated with vitamin supplementation.

120.6 Fetal Diseases Affecting the Mother

Mothers who are pregnant with a fetus affected with long-chain 3-hydroxy-acyl-coenzyme A dehydrogenase (LCHAD) deficiency have a high risk of developing preeclampsia, HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome or acute fatty liver of pregnancy (AFLP) [23]. Therefore, screening of offspring of women with AFLP and HELLP syndrome has been proposed, at least when MS/MS screening is not available, because LCHAD is potentially treatable.

120.7 Inborn Errors of Metabolism with Postnatal Onset

The clinical presentation of this group of disorders results from the accumulation of toxic compounds proximal to the metabolic defect or from deficiency in energy production. The disorders of the *intoxication* group do not interfere with the embryofetal development and present with a symptom-free interval ranging from hours to weeks, and indeed even months or years, depending on the severity of the metabolic block and/or the nature of the environmental triggers. The typical clinical features are non-specific and include poor feeding, vomiting, lethargy, irritability, seizures, and tachypnea if metabolic acidosis is present. Most of these disorders are treatable and require emergency removal of the toxin by special diet, drugs to increase clearance of toxic metabolites, and hemodialysis. The disorders of energy production are less amenable to therapy and affect primarily liver, myocardium, muscle, and brain. Although there is a significant overlap among the different conditions, from a clinical practice standpoint it is useful to classify the disorders with postnatal onset on the basis of their main clinical and/or laboratory findings at presentation [3].

120.7.1 Disorders Presenting with Metabolic Acidosis

Metabolic acidosis is very common in neonates and can be observed in a large variety of circumstances, such as infections, severe catabolic states, tissue hypoxia, and dehydration. These conditions can also be the trigger for acute decompensation in inborn errors of metabolism. The presence or absence of ketonuria is a major clinical key to the diagnosis, particularly in the neonatal period, during which significant ketonuria is highly suspicious of an inborn error of metabolism. An increased anion gap $[([Na^+]+[K^+])-([Cl^-]+[HCO_3^-])]$ greater than 25 mmol/L (normal range: 12–16 mmol/L) resulting from the accumulation of organic anions is observed in several metabolic diseases. Secondary hypoglycemia and hyperammonemia along with accumulation of organic anions result in acute encephalopathy. A number of disorders presenting with acidosis affects the catabolic pathways of the branched chain amino acids or other amino acids and are usually detected by GC/MS or MS/MS on urine specimens. A flowchart for the evaluation of infants with metabolic acidosis is presented in Fig. 120.2. The number of the disorders

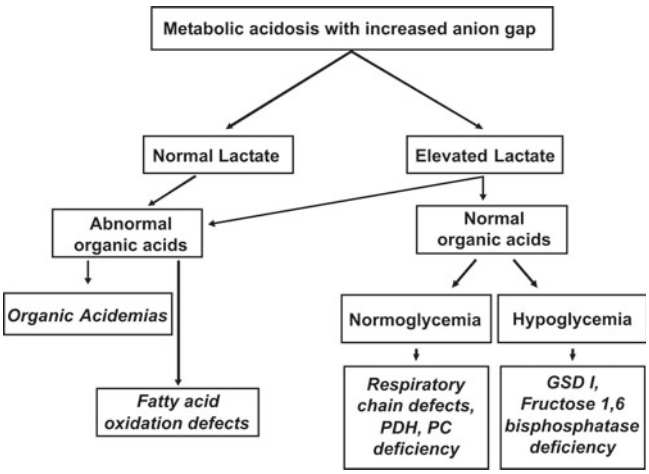

Fig. 120.2 Diagnostic approach to neonatal metabolic acidosis

of organic acid metabolism and their range of presentations is large. However, some of these conditions merit specific discussion because are relatively more common.

120.7.1.1 Propionic Acidemia and Methylmalonic Acidemia

Propionic acidemia is caused by deficiency of propionyl-CoA carboxylase which requires biotin as cofactor. Methylmalonic acidemia instead is caused by the deficiency of either the methylmalonyl-CoA mutase or the enzymes involved in the synthesis of its vitamin B12-derived cofactor, 5'-deoxyadenosylcobalamin. Patients with defects in the cofactor synthesis are responsive to vitamin B12 treatment. In most cases of both propionic acidemia and methylmalonic acidemia, the onset of the disease is in the neonatal period with the non-specific feeding difficulties, vomiting, neurological manifestations such as hypotonia, lethargy, and seizures and hematological complications (Table 120.4). If not promptly and appropriately treated, patients progress into coma with brain edema and die or develop permanent brain damage. Hyperammonemia, which may be as high as in urea cycle disorders, may complicate the initial episode of decompensation. Elevated glycine in the blood is an important clue to the diagnosis (Table 120.9) which is based on the presence of characteristic organic acids in urine as detected by GC/MS (Table 120.11) and of propionylcarnitine (C3) elevation in blood by MS/MS (Table 120.12). Cell studies on leukocytes or cultured skin fibroblasts and/or DNA studies are used for diagnostic confirmation.

120.7.1.2 Multiple Carboxylase Deficiency

There are two distinct disorders resulting in multiple carboxylase deficiency: biotinidase deficiency and holocarboxylase synthetase deficiency. Biotinidase deficiency, which is far more frequent, is due to defects in the recycling and release from dietary protein of biotin, a co-factor for the human carboxylases. Whereas patients with partial deficiency (residual activity 10–30% of mean control value) usually do not develop clinical symptoms, untreated patients with profound deficiency (residual activity < 10% of mean normal value)

Table 120.11 Urinary organic acid profiles in organic acidemias

Disease	Urine metabolites
Propionic acidemia	3-Hydroxypropionic, methylcitric, tiglylglycine, propionylglycine
Methylmalonic acidemia and cobalamin defects	Methylmalonic, 3-hydroxypropionic, methylcitric, propionylglycine
Isovaleric acidemia	3-Hydroxyisovaleric, isovalerylglycine
Glutaric acidemia type I	Glutaric, 3-hydroxyglutaric, glutaconic
Glutaric acidemia type II	Glutaric, 2-hydroxyglutaric, adipic, suberic, sebacic, dodecanoic
Malonic aciduria	Malonic, methylmalonic
Ethylmalonic aciduria	Ethylmalonic, methylsuccinic
3-Hydroxy-3-methylglutarylCoA deficiency	3-Hydroxy-3-methylglutaric; 3-methylglutaconic; 3-hydroxyisovaleric, 3 methylglutaric
3-MCC deficiency	3-Methylcrotonic, 3-methylcrotonylglycine, tiglylglycine, 3-hydroxyisovaleric
β-ketothiolase deficiency	2-Methyl-3-ketobutyric; 2-methyl-3-hydroxybutyric, 3-hydroxyisovaleric, tiglylglycine, 2-Ethyl-3-hydroxypropionic
Fumarase deficiency	Fumaric
Canavan disease	N-Acetyl-aspartic
Semialdehyde dehydrogenase deficiency	4-Hydroxybutyric; 3-hydroxypropionic, 3,4-dihydroxybutyric
Glutathione synthetase deficiency	5-Oxoproline
Methylglutaconic aciduria	3-Methylglutaconic, 3-methylglutaric
Tyrosinemia type I	Succynilacetone; p-hydroxyphenylacetic, p-hydroxyphenyllactic
Barth syndrome	3-Methylglutaconic, 3-methylglutaric
Amish lethal microcephaly	α-ketoglutaric

3-MCC 3-methylcrotonyl-CoA carboxylase.

Table 120.12 Plasma acylcarnitine patterns associated with various diseases*

Acylcarnitine name	Chain-length designation	Associated abnormality	Disorder
Free carnitine	C0 (low)		Primary carnitine deficiency
Free carnitine	C0	C16 (low); C18 (low)	CPT I deficiency
Propionyl	C3		Propionic acidemia Methylmalonic acidemia Succinyl-CoA synthetase deficiency
Malonyl	C3DC		Malonyl-CoA carboxylase deficiency
Butyryl	C4		Short-chain acyl-CoA dehydrogenase (SCAD) deficiency** Ethylmalonic encephalopathy
Isobutyryl	C4		Isobutyryl-CoA-dehydrogenase deficiency**
Isovaleryl	C5		Isovaleric acidemia
Methylbutyryl	C5		Methylbutyryl-CoA dehydrogenease deficiency**
3-Hydroxybutyryl	C4-OH		3-α-hydroxyacyl-CoA dehydrogenase deficiency
3-Hydroxyisovaleryl	C5-OH		3-Methylcrotonyl-CoA carboxylase (3-MCC) deficiency** Holocarboxylase deficiency 3-Hydroxy-3-methylglutaryl-CoA lyase deficiency Biotinidase deficiency 3-Methylglutaconyl-CoA hydratase deficiency
3-Hydroxy-2-methylbutyryl	C5-OH		2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency β-ketothiolase deficiency
Glutaryl	C5-DC		Glutaric acidemia type I
Octanoyl	C8	C6; C10; C10:1	MCAD
Tetradecenoyl	C14:1	C14; C14:2	VLCAD deficiency
Palmitoyl	C16	C18; C18:1; C18:2	CPT II deficiency CACT deficiency
3-Hydroxy-palmitoyl	C16-OH	C16:1-OH; C18-OH; C18:1-OH	LCHAD deficiency Trifunctional protein deficiency

* Acylcarnitine abnormalities include elevations over normal range unless specified otherwise.
** Clinical relevance of these biochemical abnormalities is not conclusive.

may develop various neurological deficits, skin abnormalities (Tables 120.5, 120.7), and metabolic ketoacidosis early in life. Because early recognition and oral biotin supplementation (2–20 mg/day) may completely prevent clinical and neurological deficits in biotinidase deficiency, biotinidase newborn screening has been implemented in various countries. Patients with holocarboxylase synthetase deficiency present with the characteristic skin manifestations and an overwhelming illness similar to that of propionic acidemia. In this condition the activities of all carboxylases in leukocytes or fibroblasts are reduced. Various degrees of response to biotin treatment have been reported.

120.7.2 Disorders Presenting with Ketosis

Maple urine syrup urine disease is one of the most common aminoacidopathies and presents in the newborn period with neurologic deterioration and ketosis. The disease is caused by deficiency of the branched-chain α-keto acid dehydrogenase which is involved in the catabolism of the branched-chain amino acids (leucine, isoleucine, and valine). The accumulation of leucine is neurotoxic and results in progressive brain damage. Vomiting, feeding difficulties, and lethargy are common early symptoms. Seizures occur frequently especially when cerebral edema develops. The characteristic odor may be noted as soon as neurological symptoms develop but it may be absent in a patient who has not received protein for a prolonged period of time. The diagnostic plasma amino acid pattern shows an increase of branched-chain amino acids, the accumulation of alloisoleucine, which is normally not detected, and a reduction of alanine (Table 120.9).

120.7.3 Disorders Presenting with Lactic Acidosis

Increased lactic acid is an important hallmark of metabolic diseases. However, this is also increased in hypoxic-ischemic encephalopathy, sepsis, and other conditions resulting in poor

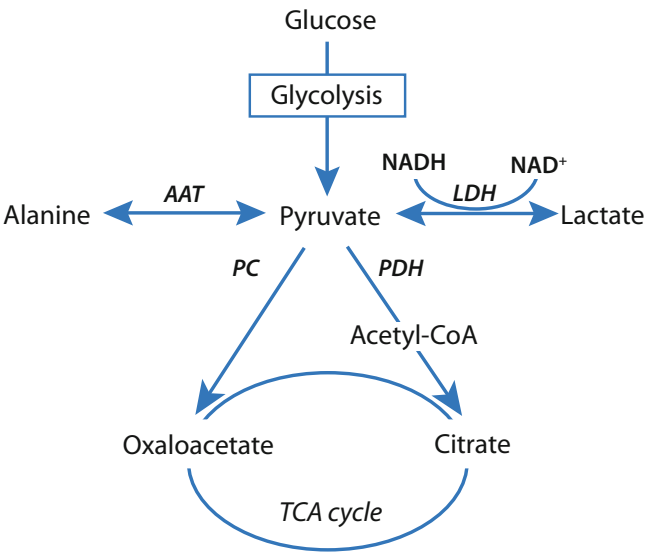

Fig. 120.3 Pyruvate metabolism. Lactate dehydrogenase (LDH), pyruvate dehydrogenase (PDH), pyruvate carboxylase (PC), alanine aminotransferase (AAT), tricarboxylic acid (TCA) cycle

vascular perfusion. Nevertheless, the most common reason for elevated lactate in blood is improper sampling technique (use of tourniquet or struggle in obtaining the sample). The measurement of pyruvate in conjunction with lactate is helpful because an increase in the plasma lactate-pyruvate ratio suggest inadequate oxygen supply. However, to obtain reliable measurements of this ratio, the plasma sample for pyruvate needs to be deproteinized at the bedside which is not practical in most clinical settings. Defects of pyruvate metabolism or of the respiratory chain result in primary lactic acidosis and should be considered in patients with persistent increase of lactic acid and normal urine organic acids. Lactic acidosis may result from an increased pyruvate production as in the case of glycogen storage disease type I or from a decreased pyruvate oxidation as a consequence of either pyruvate carboxylase (PC) or pyruvate dehydrogenase (PDH) deficiency (Fig. 120.3). In addition, it may result from defects in NADH oxidation due to mitochondrial cytopathies caused by defects of the electron transport chain. In the neonatal period, primary lactic acidoses may present with drowsiness, poor sucking, severe hypotonia, abnormal movements, seizures, respiratory distress, liver failure, optic atrophy, and fatal ketoacidotic coma.

120.7.4 Disorders Presenting with Hypoglycemia

Severe and persistent or otherwise unexplained hypoglycemia should be investigated for an underlying metabolic or endocrine cause. Recurrent, unpredictable post-prandial hypoglycemia are observed in hyperinsulinism or growth hormone deficiency and related disorders while organic acidemias or defects of gluconeogenesis (glycogen storage disease type I or fructose 1,6-bisphosphatase deficiency) usually present with associated ketoacidosis. The presence of nonglucose reducing substances in the urine is characteristic of classic galactosemia and hereditary fructose intolerance. Both diseases are generally associated with other prominent clinical problems such as liver failure. Hyperinsulinism and fatty acid oxidation disorders are the most frequent causes of hypoketotic hypoglycemia. Hyperinsulinism due to endocrine causes are described in greater detail in Chapter 121. Congenital hyperinsulinism may also be due to metabolic causes as in the case of the hyperinsulinism/hyperammonemia syndrome due to autosomal dominant mutations in the glutamate dehydrogenase (*GDH*) gene (Fig. 120.4) [24]. In this disorder, the hypoglycemia occurs with fasting but can also be triggered by protein feeding [25]. In contrast to the urea cycle disorders, the hyperammonemia is mild, stable, and without fluctuations related to fasting or protein feeding. This disorder does not present elevations of plasma glutamine which are typically seen in hyperammonemia [24]. The capacity to derive energy from mitochondrial fatty acid oxidation is critically important especially during stress and starvation. Therefore, the inherited disorders of fatty acid oxidation are an important and treatable cause of neonatal hypoglycemia.

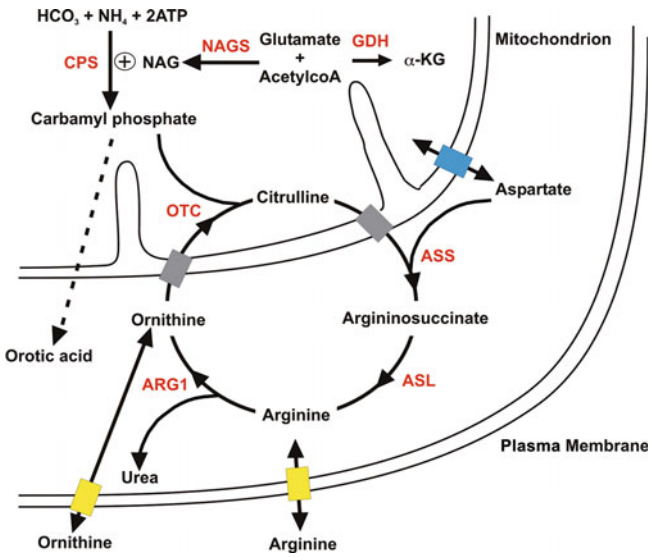

Fig. 120.4 The urea cycle. The urea cycle converts nitrogen, derived from dietary protein intake and the breakdown of endogenous protein (catabolism), into urea which can be excreted from the body. The six enzymes involved are indicated in red. The dashed *black arrow* depicts the overflow of excess carbamyl phosphate into pyrimidine synthesis and, hence, to orotic acid, which is excreted in the urine. The *gray cylinders* denote the ornithine and citrulline transporters, and the *yellow cylinders* represent the cationic-amino-acid transporter found on intestinal and kidney epithelial cells. The *blue cylinder* denotes citrin, a mitochondrial aspartate and glutamate transporter

120.7.4.1 Fatty Acid Oxidation Disorders

Mitochondrial fatty acid oxidation is a complex process involving transport of activated fatty acid molecules into the mitochondria and sequential removal of acetyl-CoA units used as fuel for the tricarboxylic acid cycle or for the production of ketone bodies. This process is critical in supplying energy during fasting and metabolic stress, and therefore patients with fatty acid oxidation defects develop hypoketotic hypoglycemia during periods of low glucose intake or intercurrent illnesses. Disorders of fatty acid oxidation may present at any age and have a broad clinical spectrum ranging from severe malformations or sudden death to almost completely asymptomatic adults [26]. They include defects of the uptake and activation of carnitine (primary carnitine deficiency); defects of the carnitine cycle [deficiency of carnitine palmitoyltransferase I (CPT I) and II (CPT II), deficiency of carnitine-acylcarnitine translocase (CACT)], required for mitochondrial entry of fatty acids; and defects of the β-oxidation enzymes. This latter group of disorders is further classified according to the length of the carbon chain of the fatty acids that accumulate and includes medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, and LCHAD deficiency. Cardiomyopathy, arrhythmias, encephalopathy, and myopathy are frequent findings in these disorders. MCAD deficiency is one of the most common inborn errors of metabolism with an incidence of 1/10,000–20,000 births [27]. Up to one quarter of the cases present in the newborn period with fasting hypoketotic hypoglycemia [28]. The treatment of MCAD is based on the avoidance of catabolism during fasting or illness and neonatal screening is effective in reducing deaths and severe adverse events [29].

As outlined above, the clinical presentation of fatty acid oxidation defects is highly variable and includes, in the case of the severe lethal neonatal variant of CPT II deficiency, a complex malformation syndrome with dysmorphic features, microcephaly, cataracts, brain and kidney anomalies, periventricular and hepatic calcifications, hypoglycemia, cardiac arrhythmias, seizures, and liver disease [5]. Expanded newborn screening has facilitated the detection of neonatal CPT II deficiency that, given its presentation, may not raise a high index of suspicion for metabolic diseases [30]. The expanded newborn screening has also allowed the identification of newborns with primary carnitine deficiency, a treatable disorder characterized by urinary carnitine losses and reduced plasma carnitine levels. The affected newborn is protected by the placental transfer and therefore, the disease onset typically occurs in infancy or childhood [31]. A few infants have also been found to have extremely low carnitine levels on newborn screening because they were born to mothers with primary carnitine deficiency who had remained asymptomatic all their lives [32].

Acylcarnitine profile analysis by MS/MS helps to establish the diagnosis of these disorders (Table 120.12). However, intermittent and reversible abnormalities can be missed if the biological specimen is collected outside the critical period. In the context of newborn screening, blood samples taken at age 48–72 hours are more likely to be diagnostic [33]. As for plasma amino acid and urinary organic acid analyses, interpretation of the results of the acylcarnitine profile is based on pattern recognition, rather than on individual abnormal values (Table 120.12). A definitive diagnosis is based on enzyme assays and/or molecular testing.

120.7.5 Disorders Presenting with Hyperammonemia

Hyperammonemia is a clinical emergency which may result in serious derangements of neurological function and structure. The degree of neurological damage is dependent on the duration of the hyperammonemia [34] and therefore, prompt recognition and intervention are important. Although the measurement of ammonia levels can be of foremost importance in making the diagnosis of a metabolic disorder, ammonia determination is unfortunately highly susceptible to artifacts resulting in false elevations. Several precautions should be followed to avoid incorrect test results: 1) the specimen must be placed on ice at the bedside; and 2) the

Table 120.13 Causes of neonatal hyperammonemia

Inherited diseases

- Urea cycle disorders
  - Carbamyl phosphate synthetase deficiency
  - Ornithine transcarbamylase deficiency
  - Argininosuccinic acid synthetase deficiency
  - Argininosuccinic acid lyase deficiency
  - N-acetylglutamate synthetase deficiency
  - Citrin deficiency
  - Lysinuric protein intolerance
  - Hyperornithinemia-hyperammonemia-homocitrullinemia (HHH) syndrome
  - Hyperinsulinism with hyperammonemia
- Other metabolic disorders
  - Propionic acidemia
  - Methylamalonic acidemia
  - Isovaleric acidemia
  - β-ketothiolase deficiency
  - Multiple carboxylase deficiency
  - Fatty acid oxidation defects
  - Pyruvate dehydrogenase deficiency
  - Mitochondrial cytopathies
  - Glutaric aciduria type II

Acquired disorders

- Any severe illness
- Transient hyperammonemia of prematurity
- Perinatal asphyxia
- Herpes simplex infection
- Total parenteral nutrition
- Vascular bypass of the liver

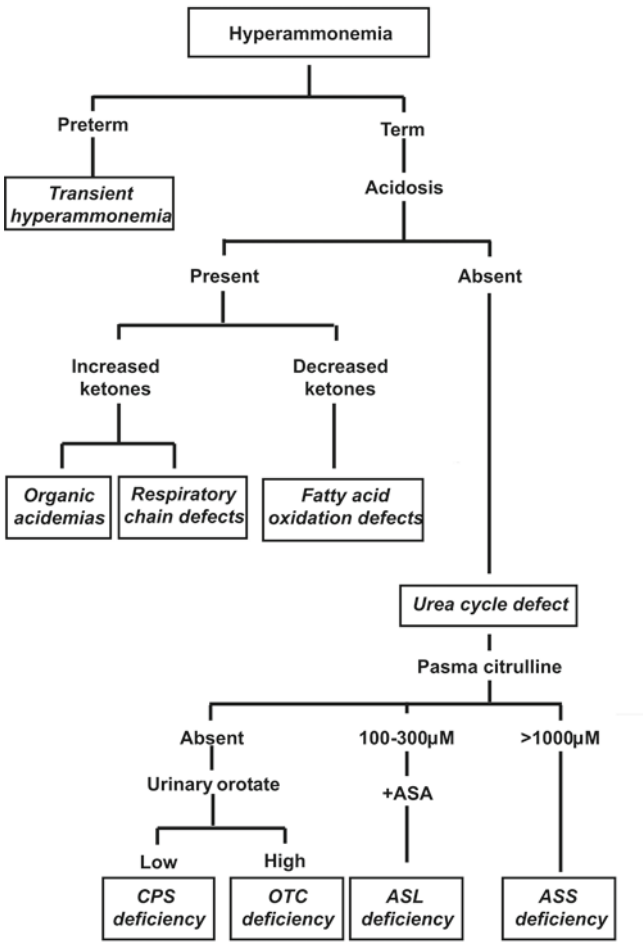

Fig. 120.5 Diagnostic approach to neonatal hyperammonemia

sample needs to be processed in a timely manner with immediate transfer to the laboratory for instant processing. Elevation of blood ammonia occurs in urea cycle disorders as well as in various other inborn errors of metabolism and in a significant proportion of premature and asphyxiated infants (Table 120.13).

The urea cycle disorders include defects of the N-acetyl glutamate synthetase (NAGS), the carbamyl phosphate synthetase (CPS), the ornithine transcarbamylase (OTC), the argininosuccinic acid synthetase (ASS), the argininosuccinic acid lyase (ASL), and arginase (ARG1) (Fig. 120.4). With the exception of OTC deficiency which is X-linked, all these disorders are inherited in an autosomal recessive pattern. Infants with urea cycle disorders appear initially normal but rapidly develop cerebral edema and the related signs of lethargy, anorexia, hypothermia, seizures, neurologic posturing, and coma. Hyperventilation is a common early finding and results in respiratory alkalosis. Hypoventilation and respiratory arrest due to an increased pressure on the brain stem may also occur [35]. The concentration of citrulline is central in the diagnostic evaluation. If citrulline is markedly elevated, the newborn has citrullinemia (ASS deficiency) and if it is low a deficiency of OTC, CPS, or NAGS is more likely. Normal or moderately elevated levels of citrulline generally indicate ASL or aginase deficiency (Fig. 120.5). Enzymatic testing on erythrocytes, fibroblasts, and liver is available along with DNA mutation detection to confirm the diagnosis. Lysinuric protein intolerance, hyperornithinemia-hyperammonemia-homocitrullinuria syndrome, and citrin deficiency are caused by defects in the transporters for the urea cycle intermediates resulting in depletion of urea cycle intermediates and decreased urea cycle activity in spite of normal urea cycle enzyme function (Fig. 120.4) [36].

120.7.6 Disorders Presenting without Hyperammonemia and without Ketoacidosis

Hypotonia and/or seizures in the absence of blood pH alteration and hyperammonemia are the main clinical findings in this group of disorders. Hypotonia is rarely isolated in patients with metabolic diseases and is commonly seen in association with other findings. Seizures are the most distinctive signal of neurological disease in the newborn period and can be caused by a broad range of systemic and central nervous system disorders. Among the metabolic defects, it is important to recognize the pyridoxine (vitamin B6)-dependent seizures because an effective treatment is available for this disorder. Pyridoxine-dependent seizures have an onset in utero or in the first hours of life [37] and are diagnosed empirically by cessation of seizures and normalization of the EEG within minutes after the intravenous injection of 50–100 mg of pyridoxine. Patients affected with pyridoxine 5' phosphate oxidase deficiency do not respond to pyridoxine but alternatively respond to the administration of pyridoxal phosphate (PLP), the active form of pyridoxine [38].

Nonketotic hyperglycinemia (also known as glycine encephalopathy) is caused by defective activity of the glycine cleavage system resulting in the accumulation of glycine in all body tissues and fluids. It typically manifests in the first hours to days of life with progressive lethargy, hypotonia, and myoclonic jerks. Mothers of affected children often report reduced fetal movements and in utero hiccups. Most affected newborns will have repeated episodes of severe and prolonged apnea, to which they succumb unless ventilatory support is provided until normal spontaneous respiration resumes after several days or weeks. Those infants regaining spontaneous respiration have an extremely poor outcome with profound mental retardation and intractable seizures [39]. Blood levels of glycine are usually increased although the elevation is occasionally modest. The laboratory diagnosis is based on simultaneous determination of CSF and plasma glycine showing an abnormal CSF-to-plasma glycine ratio (above 0.08). It is important to note that the presence

of any blood in the CSF invalidates the results. The differential diagnosis for hyperglycinemia includes disorders resulting in ketotic hyperglycinemia such as propionic acidemia and methylmalonic acidemia (Table 120.9). Organic acidemia can be distinguished from glycine encephalopathy by history, determination of urine organic acids (Table 120.11), and acylcarnitine profile (Table 120.12). Confirmatory tests include enzymatic analysis in liver tissue and/or mutation analysis [40].

Neurologic abnormalities, including seizures with neonatal onset and refractory to treatment, are also prominent in molybdenum cofactor deficiency and sulfite oxidase deficiency which can be recognized based on elevated urinary S-sulfocysteine, xanthine and hypoxanthine, reduced serum levels of uric acid, and nearly undetectable total plasma homocysteine. Seizures and encephalopathy are also features of severe methylenetetrahydrofolate reductase (MTHFR) deficiency and cerebral folate deficiency which are potentially treatable with betaine and folinic acid, respectively. The main biochemical abnormalities are homocystinuria with low or low normal plasma methionine in MTHFR deficiency and decreased CSF serum folate in cerebral folate deficiency.

120.7.7 Disorders Presenting with Liver Failure

Severe liver dysfunction in the neonate can occur in a wide variety of metabolic disorders including tyrosinemia type I, galactosemia, hereditary fructose intolerance, mitochondrial hepatopathy, fatty acid oxidation defects, sugar alcohol defects, and congenital disorders of glycosylation (Table 120.3). Neonatal hepatic failure is also a rare presentation of α_1-antitrypsin deficiency and of some bile acid synthetic defects which are described in more details in Chapters 85 and 86.

Tyrosinemia type I has been rarely described in neonates with severe liver dysfunction, hyperbilirubinemia, hypoglycemia, and hyperammonemia. The interpretation of elevated tyrosine levels is challenging because it is seen in several conditions with liver dysfunction. The diagnosis of tyrosinemia type I is confirmed by increased succinylacetone in urine or plasma.

Galactosemia will not manifest in an affected newborn until the patient is receiving galactose. Breast milk and most formulas contain lactose (a disaccharide of glucose and galactose); most soy formulas do not. Typical clinical features are hyperbilirubinemia (which may be unconjugated initially but later becomes mainly conjugated), hepatomegaly, liver dysfunction with coagulopathy, hypoalbuminemia, and hypoglycemia. Cataracts may be diagnosed as early as the disease presents in the neonatal period. If undetected the disease may progress into encephalopathy with cerebral edema, metabolic acidosis, and renal dysfunction. Patients with galactosemia have an increased risk for *E. coli* sepsis. Classic galactosemia is diagnosed by measurement of galactose-1-phosphate in serum which is increased and of the defective enzyme, galactose-1-phosphate uridyltransferase, in red blood cells. Treatment with galactose restriction in the diet results in complete resolution of the acute illness. Therefore, a galactose-free diet is recommended in case of neonatal liver failure until the diagnosis of classical galactosemia has been ruled out.

Hereditary fructose intolerance does not manifest in the newborn period unless fructose or sucrose are added to the diet.

Mitochondrial hepatopathies with deficiency of the respiratory chain enzymes have been recognized in newborns with acute neurologic impairment and liver failure [41]. In some cases, after an initial normal course, a viral infection or some other undefined event triggers hepatic and neurologic deterioration [42]. A fulminant liver failure may also be triggered by sodium valproate and therefore this medication should be avoided if there is suspicion of mitochondrial cytopathy [43]. The presence of lactic acidosis and hypoglycemia are useful diagnostic features, as is the detection of elevated tyrosine. However, these findings are rather non-specific. Respiratory chain complex analysis of liver or muscle generally shows reduced activity of respiratory chain complexes.

Sugar alcohol disorders including transaldolase deficiency and ribose-5-phosphate isomerase deficiency [44, 45] are also responsible for liver failure with neonatal onset [46, 47]. Assessment of sugar alcohol in body fluids is usually not a component of the metabolic work-up and therefore, several cases are likely to remain undiagnosed.

120.7.8 Disorders Presenting with Cholestasis

Genetic disorders are an important cause of neonatal cholestasis and α_1-antitrypsin deficiency accounts for a significant portion of these cases. α_1-antitrypsin deficiency and the inborn errors of bile acid synthesis are discussed in Chapters 85 and 86 while this section will focus on the other metabolic disorders responsible for neonatal cholestasis. Prolonged neonatal cholestatic jaundice associated with progressive hepatosplenomegaly is the most common sign in Niemann-Pick type C. Spontaneously resolving by 2–4 months of age in most patients, it may lead to liver failure in about 10% of cases. Children with this rapidly fatal neonatal cholestatic form die before the age of 6 months, without neurologic symptoms [48].

Patients with neonatal intrahepatic cholestasis caused by citrin deficiency (Fig. 120.4) have low birth weight, growth retardation, hypoproteinemia, decreased coagulation factors, and increased transaminases. Symptoms disappear by the age of 1 year with fat-soluble vitamin supplementation and lactose-free formulas or formulas containing medium-chain triglycerides. In adulthood, these patients may develop an adult-onset disease

Table 120.14 Pattern of MRI abnormalities in inborn errors of metabolism

Gray matter	White matter	Gray and white matter	Cerebellum
Cortical – Neuronal ceroid lipofuscinoses – Mucolipidoses – Lysosomal storage disorders *Striatum* – Mitochondrial diseases – Propionic acidemia – Glutaric aciduria type I – Molybdenum cofactor deficiency – Hypoglycemia *Pallidus* – Urea cycle disorders – Methylmalonic acidemia – Isovaleric acidemia – Succinic semialdheyde dehydrogenease deficiency – Pyruvate dehydrogenase deficiency – Guanidinoacetate methyltransferase (GAMT) deficiency	*Subcortical* – Mitochondrial diseases – Galactosemia – Alexander disease *Deep white matter* – Krabbe disease – Metachromatic leukodystrophy – GM_2 gangliosidosis – Peroxisomal disorders (with neuronal migration defects) – Lowe syndrome (with small cysts) – Mucolipidosis type IV – Vanishing white matter disease – Merosin deficient congenital muscular dystrophy	*Leukodystrophy and striatum* – Mitochondrial diseases – Propionic acidemia – Glutaric aciduria type I – Molybdenum cofactor deficiency – Hypoglycemia *Leukodystrophy and pallidus* – Canavan disease – Methylmalonic acidemia – L-2-hydroxyglutaric aciduria – Mitochondrial diseases – Maple syrup urine disease	– Pyruvate dehydrogenease deficiency – Leigh disease – Congenital disorders of glycosylation

with recurring episodes of hyperammonemia. The diagnosis of citrin deficiency is based on biochemical findings, including elevated ammonia, increased concentrations of citrulline and arginine, increased threonine-to-serine ratio (Table 120.9), and increased serum concentration of pancreatic secretory trypsin inhibitor. Galactose is also elevated in some cases. Conversely, patients with galactosemia can exhibit an amino acid profile resembling the profile seen in citrin deficiency [49].

120.8 Investigations for Inborn Errors of Metabolism

The first line of investigations with relatively rapid and inexpensive evaluations may provide important clues for the diagnosis of metabolic disorders in the newborn (Table 120.8). Electrolytes, blood gases, anion gap, ammonia, and lactate are useful to discriminate among the different disease groups. Blood ammonia and lactate should be determined in all neonates with unexplained lethargy and neurologic deterioration. Plasma amino acid by HPLC, urine organic analysis by GC/MS, and acylcarnitine profile by MS/MS are essential aid in the diagnosis (Tables 120.9, 120.11, 120.12). Amino acid analysis of urine is not usually performed in the evaluation of newborns and there are only a few indications for this test, such as to rule out lysinuric protein intolerance, hyperornithinemia-hyperammonemia-homocitrullinuria syndrome, and cystinuria. Most sick neonates undergo lumbar puncture for CSF collection as part of sepsis evaluation. In the context of a patient with encephalopathy, it is recommended to save an aliquot of CSF for lactate and amino acid determination.

Neuroimaging studies can provide important clues for the diagnosis of metabolic disorders. Patterns of brain involvement can be useful in the diagnostic process [50]. The first important assessment is to establish whether the disease involves primarily gray matter, primarily white matter, or both gray and white matter (Table 120.14). Moreover, proton magnetic resonance spectroscopy (MRS) can be helpful for diagnosis and monitoring in a selected number of disorders.

The diagnosis of inborn errors of metabolism should be confirmed by enzyme analysis and/or DNA tests. The choice

Table 120.15 Specimens to be collected in the event of death when metabolic disease is suspected

Plasma (> 2 mL)	Heparinized, separated and frozen at –80°C
Blood	Spots on filter paper for MS/MS
Urine (5-20 mL)	Deep frozen in a plain tube
CSF	1 mL stored at –80°C
Sample for DNA	Blood anticoagulated with EDTA and frozen at –80°C*
Skin for fibroblast culture	Taken with sterile precautions into tissue culture medium and stored at 4°C
Liver	Snap frozen (–80°C) for histochemistry/enzymology
Muscle	Snap frozen (–80°C) for histochemistry/enzymology

* Skin fibroblasts or other tissues may also be used for DNA extraction.

Table 120.16 Medications for acute decompensation in inborn errors of metabolism

Condition	Medication	Route	Recommended dose
Organic acidurias Fatty acid oxidation defects	L-carnitine	IV, PO	100 mg/kg/day
Hyperammonemia	Na benzoate	IV	250 mg/kg bolus over 2-4 hours followed by infusion of 250 mg/kg over 24 hours
Urea cycle disorders	L-arginine	IV	200-600 mg/kg followed by infusion of 250 mg/kg over 24 hours
Methylmalonic acidemia	Vitamin B12	IV, IM	1–2 mg/day
Pyridoxine-responsive seizures	Pyridoxine	IV	100 mg
Pyridoxal phosphate-responsive seizures	Pyridoxal phosphate	PO	30 mg/kg/day
Cerebral folate deficiency	Folinic acid	IV, PO	5–15 mg/day
Maple syrup urine disease	Thiamine	IV	25–100 mg
Propionic acidemia, multiple carboxylase deficiency	Biotin	IV, PO	10 mg

of one or both of these tests is usually dictated by the availability of laboratory offering these analyses. Enzyme tests can be performed on different tissues (blood, liver, fibroblasts) depending on the specific disease. DNA tests instead are performed on DNA extracted from blood. Biochemical methods (detection of metabolites in amniotic fluid and enzyme assays using cultured cells) as well as DNA analysis (mutation detection) are used for prenatal diagnosis. Helpful resources to localize a laboratory for a specific biochemical or molecular test are GeneTests (www.ncbi.nlm.nih.gov/sites/GeneTests/) or Orphanet (www.orpha.net/).

Inborn errors of metabolism can result in sudden unexplained death. Under these circumstances, it is particularly important to perform a *metabolic autopsy*, skeletal survey, and collect appropriate biologic samples to perform further diagnostic tests (Table 120.15). The findings of these investigations may have important implications for genetic counseling.

120.9 Treatment of the Acute Decompensation

The management of the acutely ill neonate must be initated early and must be aggressive. The conventional approach to the treatment of inborn errors of metabolism involves the following basic principles: 1) restriction of upstream essential nutrients to prevent intoxication; 2) supplementation of downstream nutrients to prevent secondary deficiency; and 3) stimulation of alternative routes for disposal of precursor metabolites.

During the acute phase of decompensation, treatment to keep catabolism to a minimum and remove toxic metabolites is required in most disorders. These measures can be started before a precise diagnosis is known. First, any nutrient that may have precipitated the illness, such as galactose or protein, should be halted and an intravenous high energy intake made up to 10% dextrose (~10 mg/kg/min) with mantainance electrolytes should be started. The need to provide adequate glucose may require the use of concentrated solutions through a central line which can be useful in case extracorporeal dialysis is needed. With high rates of glucose infusion, blood glucose concentrations should be monitored and if hyperglycemia occurs, insulin may be started. In this context, insulin has the additional advantage of promoting anabolism. Intravenous fluid should be administered with caution to avoid fluid overload and cerebral edema which may occur in maple syrup urine disease and urea cycle disorders. Metabolic acidosis can be corrected by sodium bicarbonate administration often required in large doses.

Hypoventilation due to cerebral depression is the most common reason for poor tissue oxygenation and most affected newborns require mechanical ventilation. Many patients with metabolic decompensation may develop sepsis which may aggravate the catabolism and results in therapeutic failure. Therefore, an aggressive search and treatment of infections are crucial for a successful intervention. Sodium benzoate is used to treat the hyperammonemia (Table 120.16). Carnitine is effective for the elimination of some toxic metabolites and it may be given even before a specific diagnosis is known. In the presence of severe lactic acidosis or unexplained metabolic acidosis, biotin and intramuscular vitamin B12 treatment can be initiated until multiple carboxylase deficiency and methylmalonic acidemia have been excluded (Table 120.16). Sometimes the metabolic derangement cannot be controlled by the approaches outlined above. Given that neurological complications are related to the concentration and duration of the exposure to the toxic metabolites, such as ammonia and leucine, more aggressive therapies such as hemodialysis are required.

It is highly recommended that a neonate who is suspected to have an acute decompensation due to a metabolic disorder be immediately referred to a newborn intensive care unit with expertise in the treatment of metabolic patients.

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121

Endocrine Diseases of Newborn

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121.1 Hypopituitarism

Congenital hypopituitarism results from a deficiency of any or all of the hormones secreted by the anterior pituitary gland (adreno-corticotropic hormone [ACTH]; growth hormone [GH]; thyroid-stimulating hormone [TSH]; luteinizing hormone [LH]; follicle stimulating hormone [FSH]; prolactin [PRL]) or by the posterior pituitary gland (antidiuretic hormone [ADH]). It is uncommon. The annual incidence ranges from 1 to 42 new cases per million and a prevalence of 300–455 cases per million [1].

121.1.1 Etiology

The incidence variability depends on the heterogeneity of cases. Some cases have a genetic etiology, others with lesions in the sellar region have been attributed to traumatic events or perinatal complications (cesarean section, breech, prolonged or rapid delivery, and low Apgar score) [2]. It is, however, difficult to determine whether perinatal insults lead to hypopituitarism because of traumatic disruption of the pituitary stalk, or whether hypopituitarism with structural hypothalamo-pituitary defects [3] results in an increased prevalence of perinatal complications.

Congenital hypopituitarism may be due to hypothalamic dysplasia or specific releasing hormone deficiencies (corticotropin-releasing hormone [CRH]; growth-hormone releasing hormone [GHRH]; gonadotropin-releasing hormone [GnRH]; thyrotropin-releasing hormone [TRH]). Other causes are due to anterior pituitary dysplasia or specific anterior pituitary deficiencies. Posterior pituitary deficiency may be familial (X-linked or autosomal dominant), idiopathic or secondary to inflammatory events (e.g., meningitis), disseminated intravascular coagulation (DIC), intraventricular hemorrhage, trauma/asphyxia and maternal drugs (e.g., lithium). Congenital rubella and toxoplasmosis have also been implicated.

Recently, mutations in a several genes encoding transcription factors that are implicated in normal hypothalamic-pituitary (H-P) development have been linked both with hypopituitarism in humans and with structural abnormalities on neuro-imaging. Mutations in genes that are implicated in somatotrope proliferation and GH secretion, such as *GH1* and *GHRHR*, are also associated with GH deficiency and with magnetic resonance imaging (MRI) abnormalities. These transcription factors and signaling molecules are expressed either exclusively in the developing pituitary or also in structures such as the hypothalamus and forebrain. A mutation in any one of these can lead to either a pituitary-specific phenotype (Table 121.1) or a broader clinical presentation such as septo-optic dysplasia, agenesis of the corpus callosum, persistent septum pellucidum, central cleft lip/palate, anencephaly/holoprosencephaly, familial pituitary hypoplasia and Wolfram syndrome.

121.1.2 Clinical Presentation

Congenital hypopituitarism must be suspected in any child presenting hypoglycaemia and dysmorphic features with midline defects, prolonged jaundice, poor weight gain, hypothermia, cryptorchidism and/or micropenis in a male infant. Early diagnosis of this condition may prevent impairment of cognition, poor growth and metabolic abnormalities.

In anterior hypopituitarism, there may be optic nerve hypoplasia/dysplasia. Babies tend to be small, sometimes severely growth restricted. In posterior hypopituitarism, polyhydramnios may be followed by postnatal dehydration, weight loss, irritability, hypernatremia and seizures. In

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 121.1 Clinical phenotypes, mode of inheritance, and MRI presentation in pituitary hormone deficiencies involving gene alterations. Modified from [3]

Gene	Inheritance	Deficiency	Anterior hypophysis	Posterior hypophysis	Other abnormalities
HESX1	AR or AD	GH, (TSH, LH, FSH, ACTH, PRL variable)	Hypoplasic	Ectopic	Septo-optic dysplasia
PROP1	AR	GH, TSH, LH, FSH, (ACTH variable)	Enlargement with waxing and waning and eventual hypoplasia	Normal	Transient AP hyperplasia
LHX3	AR	GH, TSH, LH, FSH, PRL	Variable	Normal	Abnormalities of head rotation
LHX4	AD	GH, TSH, (LH, FSH, ACTH variable)	Hypoplasic	Variable	Cerebral malformations
POU1F1 (*PIT1*)	AR or AD	GH, TSH, PRL	Hyperplasia and later hypoplasia	Sometimes ectopic	–
SOX2	AD	HH, variable GHD	Hypoplasic	Normal	An/microphtalmia, esophageal atresia, genital tract abnormalities, hypothalamic hamartoma, sensorineural hearing loss, diplegia
SOX3	XL	IGHD or CPHD	Hypoplasic	Ectopic	Mental retardation
TBX19 (*T-PIT*)	AR	ACTH	Normal	Normal	Neonatal
GH1		GH	Empty sella, hypoplasia	Normal	
-IA (del/severe mutations)	AR				IA no GH and GH Ab on treatment
-IB (splice site, missense mutations)	AR				IB low detectable GH, no Ab
-II (splice site, splice site enhancers, missense muts)	AD				II less severe short stature
GHRHR	AR	High GH	–	Normal	–

AR autosomal recessive, *AD* autosomal dominant, *XL* X-linked, *Ab* antibodies.

breast-fed babies, the clinical features are similar but of more gradual onset. In patients with both arginine vasopressin and ACTH deficiency, diabetes insipidus, characterised by polyuria and polydipsia, may be masked by ACTH deficiency – cortisol is essential for the excretion of a water load. Treatment with hydrocortisone may unmask diabetes insipidus (DI).

121.1.3 Investigations

Biochemical/hormonal Measurements

First check blood glucose levels. If there is severe or symptomatic hypoglycaemia, the levels of GH, cortisol and ACTH should be measured. If found to be high because of stress (e.g., difficult venous cannulation), then pituitary function tests may be avoided. Baseline TSH, and free thyroxine (fT4) or LH/FSH levels may also be useful as they should be relatively high in the first days of life.

Tests of pituitary function should be undertaken under the direction of an endocrinologist. A glucagon stimulation test will assess both GH and adrenal axes, but late hypoglycemia may be dangerous. An ACTH stimulation test is safe but only indirectly evaluates ACTH. A CRH test measuring cortisol and ACTH response may be more useful. A gonadotropin-releasing hormone (GnRH) test to stimulate LH and FSH may be helpful for later management of puberty and fertility.

The diagnosis of ADH deficiency is made by monitoring weight, fluid balance, and urine and plasma osmolalities (low urine and high plasma osmolality).

Imaging

Cranial ultrasound and MRI scan (hypothalamus and pituitary gland are not visible on ultrasound scan) may reveal associated structural anomalies of the brain, hypothalamic-pituitary axis, and the optic nerves (Table 121.1).

Genetic Analysis

The relevant genetic analysis should be undertaken in patients with multiple pituitary hormone deficiencies, a family history of hypopituitarism or consanguinity or with associated anatomic abnormalities (Table 121.1).

121.1.4 Management

Acute Interventions

Correction of hypoglycemia is the first step. Substitution therapy with deficient hormones must be undertaken as soon as possible. ACTH, TSH and ADH deficiencies need replacement with hydrocortisone (8–12 mg/m^2/day orally, in three divided doses), thyroxine (8–12 μg/kg/day, orally once a day) and vasopressin (0.5–2.0 mU/kg/h as a continuous IV infusion) or DDAVP (0.25–10 μg 12 h intranasally, or 0.02–0.2 μg 12 h intravenously, according to response), respectively. When hypoglycemia is caused by GH deficiency, then subcutaneous hGH at 7 mg/m^2/weekly dose may be useful.

Discharge Instructions

Intramuscular injections to take home (hydrocortisone hemisuccinate, 70 mg/m^2/8 h) need to be prescribed in case of vomiting or profuse diarrhea. In the case of intercurrent illness, instructions from a pediatric endocrinologist should be given to double (non-febrile illness) or treble (febrile illness) the oral hydrocortisone doses. A steroid card should be provided.

Parents should be instructed in the use of glucose monitoring (sticks) and the management of hypoglycaemia (1 μg s.c. glucagon injections).

In presence of a micropenis, the use of testosterone injections (25 mg/IM monthly, for 3 months) or dihydrotestosterone gel (0.2–0.3 mg/kg topically once a day for 3–4 months) during the first 6 months of life may help further virilization.

Optic nerve involvement mandates ophthalmic referral.

Long-Term Follow-up

Generally, the anterior pituitary deficiencies need a permanent substitution treatment, while the posterior pituitary may recover. Genetic counseling should be provided for inherited causes of hypopituitarism.

121.2 Adrenal Insufficiency

Reduced cortisol and/or aldosterone production may be the consequence of primary cortical adrenal deficiency or damage. Isolated cortisol insufficiency may also be a consequence of pituitary/hypothalamic ACTH/CRF deficiency (secondary/tertiary adrenal insufficiency, respectively). The clinical presentation will differ according to the underlying cause.

121.2.1 Adrenal Development from Fetal to Postnatal Adrenal Gland

Coelomic epithelial cells of mesodermal origin on the urogenital ridge give rise to the kidney, gonadal and adrenal cortical structures (Fig. 121.1). The fetal adrenal cortex separates from the other structures and differentiates into a larger inner fetal zone, which produces DHEA and DHEA-S from early gestation, but 3-β-HSD expression is absent after 12 weeks. After 9 weeks' gestation, there is a thinner outer definitive zone, which is quiescent until late gestation. An intermediate transitional zone appears after 24 weeks' gestation and contains the enzymes for cortisol production (expression of 3-β-HSD activity). Shortly after birth, the fetal zone regresses. By 6 months of age the definitive (adult) adrenal cortex consists of the mineralocorticoid-producing zona glomerulosa and the glucocorticoid-producing zona fasciculata. The zona reticularis does

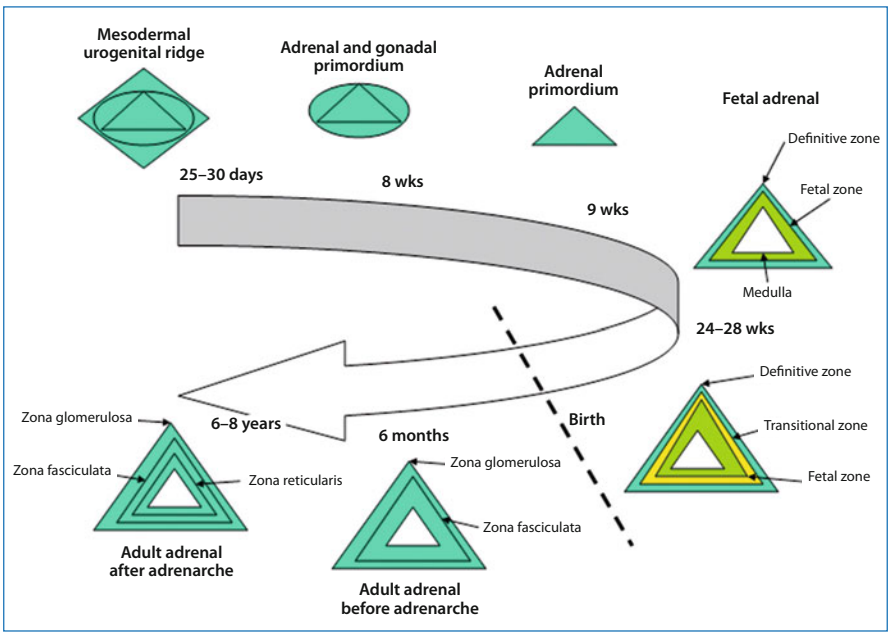


Fig. 121.1 Scheme of temporal adrenal development in humans. Modified from [8]

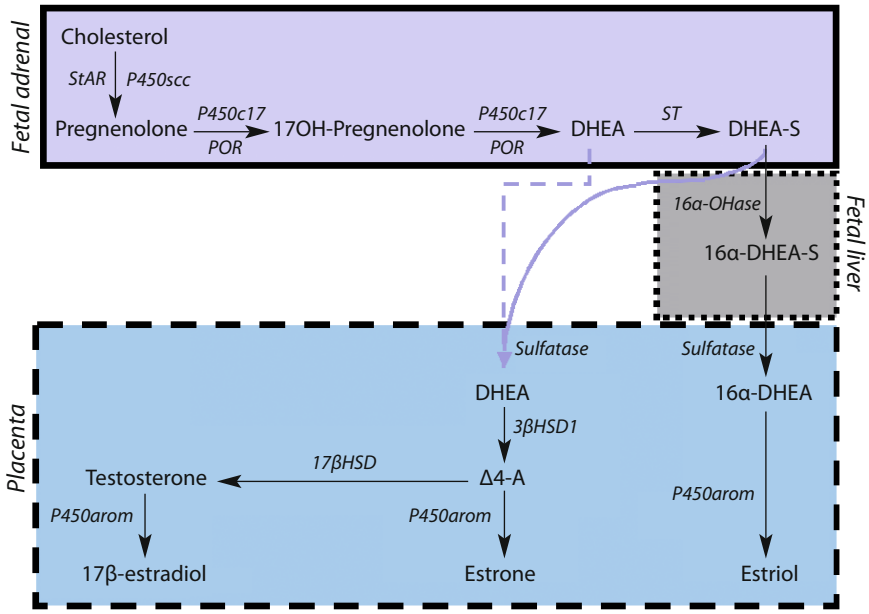


Fig. 121.2 Steroidogenesis of the fetal-adrenals/-placental unit. DHEA and its sulphate are the main products of the fetal adrenal cortex after 12 weeks' gestation in humans. DHEA-S is produced from the adrenals and converted to oestriol in the placenta through previous 16α-hydroxylation in the fetal liver, or converted directly to oestrogens in the placenta

not appear until the end of year 3 and only by around 6 years of age starts to produce adrenal androgens (adrenarche) [4].

Fetal DHEA-S production is important because it is either 16α-hydroxylated in the fetal liver before being converted to estriol (90% of the maternal circulation) or directly converted to estrone and estradiol in the placenta (50% of the maternal circulation) (Fig. 121.2).

The fetus seems to be protected from high levels of cortisol in utero because cortisol is converted to biologically inactive cortisone through 11β-HSD2 activity in placental and fetal tissues. However, transient expression (weeks 7–12 of gestation) of 3β-HSD2 in the fetal adrenal has been recently demonstrated [5] and serves to protect female fetuses from genital virilization. It enables the fetal adrenal to produce cortisol by 8 weeks of gestation and activates the negative feedback on pituitary ACTH which in turns reduces the early production of androgens in the critical time window for genital development [6].

121.2.2 Molecular Basis of Adrenal Insufficiency

More than 65 genes are thought to be involved in adrenal development, including genes coding for steroidogenic enzymes, signaling molecules, transcription and growth factors, regulators of cell cycle and angiogenesis, and extracellular matrix proteins [7, 8]. The exact role of most of these factors remains unclear.

Studies of patients with adrenal insufficiency due to gene mutations have revealed that transcription factors such as *GLI3*, *SF1* and *DAX1* have a central role in the first steps of adrenal formation. Adrenal differentiation requires ACTH stimulation and signaling through biosynthetic steps involving POMC, PCI, TPIT, MC2R and ALADIN, all of which determine adrenal hypoplasia when mutated. Mutations in some of the steroidogenic enzymes cause congenital adrenal hyperplasia, autosomic recessive inherited diseases impairing both cortisol and/or aldosterone synthesis.

121.2.3 Etiology

121.2.3.1 Congenital

Congenital adrenal insufficiency comprises a group of disorders including: 1) defects of adrenal development; 2) disordered adrenal steroidogenesis; 3) degenerative metabolic disorders affecting the adrenals (e.g., adrenoleukodystrophy); 4) disturbed endocrine communication between the pituitary gland and the adrenals. Table 121.2 summarizes the most frequent inherited conditions associated with neonatal adrenal insufficiency.

Primary Forms

Adrenal congenital hypoplasia (ACH) can present as different clinical forms of primary adrenal insufficiency:

1. An autosomal recessive form with a characteristic miniature adult adrenal morphology, with small glands with a permanent cortical zone but a diminished fetal zone. The molecular basis of this form is still unclear, but lack of late gestational or early postnatal stimulation by pro-opiomelanocortin (POMC) peptides or ACTH signaling is possible;

Table 121.2 Congenital causes of adrenal insufficiency (AI) in the newborn

Disorder	Gene/protein type	Chrom. location	Inheritance (OMIM)	Global [1]	Partial [2]	External genitalia	Other associated features
Congenital hyperplasia							
Lipoid CAH	***StAR***/shuttle protein, ***CYP11A1***/enzyme	8p.11.2, 15q23-24	AR (201710)	Yes	–	Female or ambiguous	–
3βHSD3 type 2 deficiency	***HSD3B2***/ enzyme	1p13.1	AR (201810)	Yes	–	Female or ambiguous	–
P450c21 deficiency	***CYP21A2***/enzyme	6p21.3	AR (201910)	SW	SV [a]	Male or ambiguous	–
P450c11 deficiency	***CYP11B1***/enzyme	8q21.22	AR (202010)	–	Yes [a]	Male or ambiguous	Hypertension
POR deficiency	***POR***/*CYP* electron donor	7q11.2	AR (201750)	–	Yes [a]	Male or ambiguous	Mixed features of 21-OH,17-OH, aromatase deff.
Congenital hypoplasia							
X-linked ACH	***DAX1 (NROB1)***/ TF(NR)	Xp21.3-21.2	X linked (300200)	Yes	–	Normal male	Hypogonadotropic hypogonadism
Autosomal recessive ACH	Unknown	–	AR	Yes	–	Normal	
SF1 linked ACH	***SF1 (NR5A1)***/ TF(NR)	9q33	AD/AR (184757)	Yes	–	Male ambiguity to sex reversal	Sometimes no AI and DSD only
IMAGe syndrome	Unknown	–	X linked	Yes	–	Cryptorchidism, micropenis; normal F	IUGR, metaphyseal dysplasia
Mineralocorticoid deficiency							
Aldosterone syntetase deficiency	***CYP11B2***/enzyme	8q21-22	AR (124080)	–	Yes [b]	Normal	–
Pseudo-hypoaldosteronism	***NR3C2***/TF(NR)	4q31.1	D (177735)	–	Yes [b]	Normal	Only kidney
	EnaC-α, -β, -γ	12p13, 16p13-12	AR (600228; 600760-1)		Yes [b]	Normal	Also other organs
ACTH insensitivity syndrome							
Familial glucoc. deficiency S.	***MC2R***/(MR); ***MRAP***/(AP)	18p11	AR (607397)	–	Yes [a]	Normal	–
Triple A syndrome	***AAAS***/ regulatory pr.	12q13	AR (231550)	–	Yes [a]	Normal	Achalasia, alacrimia
ACTH deficiency							
Isolated	***TPIT***	1q23-24	(201400)	–	Yes [a]	Normal	–
Combined	PROP1, HESX1, LHX4	5q, 3p21.1-21.2, 1q25	(601538, 601802, 602146)	–	Yes [a]	Normal, microphallus	HH, GH deficiency, hypothyroidism
Degenerative metab. disorders							
Adrenoleukodistophy X-ALD	***ABCD1***/TF	Xq28	X linked (300100)	Yes	–	Normal	X-ALD also in contiguous gene S.:
Neonatal ALD	***PEX10***, ***PEX13***, ***PEX1***, ***PEX5***, ***PEX26***	12p13.3, 7q21-q22, 1p36.32, 2p15, 22q11.21	AR (202370)	Yes	–	Normal	GK S., Duchenne, AHC
Mitocondrial disease	**Mt DNA**	–	–	Yes	–	Normal	
Wolman disease	***LIPA***/enzyme	10q24-25	AR (278000)	Yes	–	Normal	Calcified adrenals
Smith-Lemli-Opitz syndrome	***DHCR7***/enzyme	11q12.13	AR (270400)	Yes	–	Ambiguous	

[1] Cortisol and aldosterone deficiency.
[2] [a] Cortisol deficiency; [b] Aldosterone deficiency.
OMIM Online mendelian inheritance in man, *TF* transcription factor, *NR* nuclear receptor, *MR* membrane receptor, *AP* accessory protein.

2. An X-linked cytomegalic form, in which the normal three zone architecture of the adrenal cortex is absent and is replaced by large, vacuolated cells resembling fetal adrenocortical cells. It is caused by mutations in the *DAX1* gene that also cause hypogonodotropic hypogonadism and impaired spermatogenesis [9];
3. ACH, caused by mutations in the *SF1* gene, can be dominantly or recessively inherited. It is associated with adrenal failure and XY sex-reversal with the presence of müllerian structures. Since the developing adrenals appear less *SF1* dose-dependent than the testes, not all affected patients may have adrenal insufficiency;

4. IMAGe syndrome (Intrauterine growth restriction, Metaphyseal dysplasia, Adrenal hypoplasia, Genital abnormalities) comprises a cluster of abnormalities (see Table 121.2) that suggest it may be caused by a novel gene involved in the development of bone, adrenal cortex, and anterior pituitary [10].

Congenital adrenal hyperplasia (CAH) (*CYP21A2* mutations account for more than 90% of cases) is one of the most frequent forms of cortisol and aldosterone deficiency in the newborn. Other rarer enzymatic defects in this pathway (Table 121.2) can cause varying degrees of virilization of females and undervirilization of males. Patients with P450 oxidoreductase (*POR*) mutations, a recently described form of CAH, may be at risk of adrenal insufficiency and Addisonian crisis, especially at times of severe febrile illness or major surgery. In these conditions, the hypertrophic adrenals maintain their histological structure. Gene analysis, especially for 21-hydroxylase deficiency, makes it possible to perform prenatal diagnosis and treat female affected fetuses (see also Chapter 123).

In isolated hypoaldosteronism (*CYP11B2* deficiency, *CMO I* and *CMO II* forms) and pseudohypoaldosteronism (PHA, affecting the kidney only or where there is multi-organ resistance to mineralocorticoids), the adrenal cortex is normal. Both *CYP11B2* deficiency and systemic pseudohypoaldosteronism (PHA) inheritance are autosomal recessive disorders, while the renal PHA form is autosomal dominant [11]. An acquired form of PHA also exists and it is important to recognize it during the neonatal period when an urinary tract malformation is detected by prenatal ultrasound. In the case of obstructive uropathy, the risk of salt loss is very high when the cause of obstruction is treated by surgery.

Adrenoleucodistrophy is an X linked (X-ALD) severe neurodegenerative disorder causing progressive demyelinization of the central and peripheral nervous system, adrenal insufficiency, and accumulation of very-long-chain fatty acids in plasma, fibroblasts and tissues. It is caused by impaired beta-oxidation in peroxisomes due to mutations in the *ABCD1* gene. The onset generally does not occur below 3 years of age, but a contiguous *ABCD1 DXS1357E* deletion syndrome (CADDS) has recently been described with neonatal onset [12]. Its phenotype is similar to that of either peroxisomal biogenesis disorders (PBD) or single-enzyme deficiencies (SED) in the peroxisomal beta-oxidation pathway (acyl CoA oxidase deficiency and bifunctional protein deficiency), which also accumulate very-long-chain fatty acids, but are transmitted in an autosomal recessive fashion and present as neonatal onset ALD (Table 121.2). Neonatal screening by tandem mass spectrometry can be used for early diagnosis, reducing morbidity due to these conditions [13].

Secondary Forms

ACTH deficiency may recur as an isolated feature (IAD) or as part of a multiple pituitary hormone deficiency syndrome (Table 121.2). IAD is rare and may occur in the neonatal period, but also in later childhood. After consideration of candidate genes (*POMC*, *PC1*, *CRH* and its receptor *CRH-R1*), only mutations in *TPIT* have been found in about 25% of the patients with the isolated, early onset form. Multiple pituitary hormone deficiencies (MPHD) may result from abnormal hypothalamic-pituitary development caused by anomalous expression of the transcription factors reported in Table 121.2. ACTH insensitivity syndromes comprise a group of disorders which all manifest as familial glucocorticoid deficiency (FGD). The type 1 (FGD1) depends on autosomal recessive mutations in the G-protein-coupled ACTH receptor (melanocortin receptor 2, MC2R), while the type 2 (FGD2) is caused by mutations in the gene encoding *MC2R* accessory protein (*MRAP*) [14]. Other forms of FGD (type 3) still remain without corresponding identified gene mutations. ACTH resistance is one of the three components of the Triple A syndrome (Achalasia-Addisonianism-Alacrima, the Allgrove syndrome), caused by mutations in the *AAAS* gene, encoding a protein named ALADIN, and which manifests itself during the first decade.

121.2.3.2 Acquired

AIDS, meningococcus, adrenal hemorrhage (Waterhouse-Friderichsen), maternal Cushing's disease/syndrome, adrenal suppression following antenatal steroid therapy to the fetus or to the mother, may all cause severe adrenal insufficiency in neonates. The use of dexamethasone postnatally has been largely abandoned because of likely effects on brain growth and more physiological doses of hydrocortisone are currently being used in the context of clinical trial for treatment of the controversial relative adrenal insufficiency [15].

121.2.4 Clinical Presentation

Generally, primary congenital adrenal insufficiency may present with hypoglycemia, cardiovascular collapse, hypotension, ambiguous genitalia, skin pigmentation and hyponatremia/ hyperkalemia. Patients with congenital adrenal hypoplasia, steroidogenic defects or pseudohypoaldosteronism present with clinical signs of salt wasting within the first 20 days after the birth. They may have failure to thrive, anorexia, vomiting, and severe hyponatremic/hyperkalemic dehydration. Skin hyperpigmentation, typical for the forms with reactive ACTH overproduction (congenital adrenal hyperplasia/hypoplasia, familial isolated glucocorticoid deficiency, adrenoleukodystrophy) may color genitalia, umbilicus, nipples and axillae. Other symptoms at onset, mainly in neonates with severe glucocorticoid deficiency (congenital adrenal hyperplasia, familial isolated glucocorticoid deficiency), may be hypoglycemia with possible ketosis, due to gluconeogenic substrate deficiency.

Hyperpigmentation and salt craving are not observed in patients with secondary adrenal insufficiency. Unless there is a history of recent glucocorticoid therapy, secondary adrenal insufficiency is usually associated with signs of other pituitary hormone deficiencies such as hypoglycemia, growth failure, secondary hypothyroidism, and/or diabetes insipidus (polyuria and polydipsia).

121.2.5 Diagnostic Evaluation

When the adrenal failure is not extremely severe, a pre-treatment blood sample for cortisol, electrolyte, glucose, ACTH levels, plasma renin activity and aldosterone level should be taken. A further diagnostic evaluation under the supervision of a pediatric endocrinologist may need an ACTH test (0–60 mins, half standard dose: 125 μg IV or low dose: 0.5–1 μg/1,73 m^2) to assess the adrenal response. A normal cortisol response is a rise of 200 nmol/L from baseline, although equivocal results may occur during the neonatal period. A peak 17OH-progesterone of more than 150 nmol/L is suggestive of 21-hydroxylase deficiency (for other specific steroidogenic defects see Table 121.2 and Chapter 123, Table 123.4). Urine steroid profile may be useful for AHC or CAH differentiation and adrenal ultrasound scan for hemorrhage or hypertrophy. Aldosterone levels are generally low in salt-wasting congenital adrenal hyperplasia/hypoplasia. When isolated aldosterone deficiency is suspected, very high levels of the hormone indicate possible pseudohypoaldosteronism. Plasma renin activity (PRA) levels are elevated when salt wasting is latent or manifest due to aldosterone deficiency or resistance. In aldosterone steroidogenic defects, the PRA/aldosterone ratio is elevated even when mineralocorticoid levels are normal.

121.2.6 Treatment

For the standard treatment of glucocorticoid and/or mineralocorticoid deficiencies, see Chapter 123. Secondary adrenal insufficiency or familial glucocorticoid deficiency do not need mineralocorticoid substitution. Patients with isolated aldosterone deficiency do not need glucocorticoids and milder forms may be treated by the simple addition of salt to the diet, obviating the need for mineralocorticoid substitution. In patients with pseudohypoaldosteronism, where mineralocorticoid treatment is ineffective, dietary supplementation with sodium chloride is the only possible treatment.

In acute adrenal crisis, an initial hydrocortisone IV bolus of 60–70 mg/m^2 is recommended, followed by 60–70 mg/m^2 per day divided into four IV doses. For restoration of intravascular volume, we recommend an infusion of 5% glucose and 0.9% saline solutions (120–150 mL/kg/24 hrs). The combination of saline and large doses of hydrocortisone maintains mineralocorticoid function during the first hours of treatment. Fludrocortisone (0.05–0.2 mg/day in two divided doses) may be administered orally; intramuscular administration of 1–2 mg/ twice a day of corticosterone acetate is a less comfortable way of achieving mineralocorticoid replacement. Additional glucose should be administered parenterally when hypoglycemia is severe.

121.3 Hyponatremia and Hypernatremia

Total body water and osmolality is regulated by the renal action of antidiuretic hormone (ADH), the renin-angiotensin-aldosterone system, norepinephrine and by the thirst mechanism. Abnormalities in water balance are manifested as sodium disturbances. Mild to moderate hyponatremia (plasma sodium > 125–134 < mmol/L) and severe hyponatremia (<125 mmol/L) are found in about 15–30% and 1–4% of patients, respectively. It is useful to distinguish between water overload (excessive intake or inadequate excretion) and sodium depletion (excessive salt loss). Common causes of hyponatremia are listed in Table 121.3. Hypernatremia

Table 121.3 Causes of hyponatremia and hypernatremia in the newborn

Hyponatremia
Water overload
– Iatrogenic: excess maternal (prenatal) or neonatal i.v. fluid administration
– Renal failure
– Syndrome of inappropriate antidiuretic hormone secretion (SIADH)
Salt depletion
– Immaturity
– CAH, AHC
– Renal impairment: polyuric phase of acute tubular necrosis (ATN), congenital nephrotic syndrome, Bartter syndrome, congenital renal abnormality (previous polydramnios)
– Gut loss: secretory diarrhea, stoma output following gut resection, congenital chloride diarrhea
– Iatrogenic: repeated cerebral spinal fluid removal by ventricular taps
– Aldosterone biosynthetic defect or resistance
Hypernatremia
Water depletion
• Inadequate fluid intake:
– milk intake: frequent in breastfeeding mothers at first pregnancy
– i.v. fluid infusion: e.g. estreme preterm infant with high insensibile percutaneous or respiratory tract loss
• Excessive fluid loss:
– urine: diabetes insipidus (DI), renal concentrating defect, osmotic diuresis secondary to glicosuria
– gut: vomiting or naso-gastric aspirates, secondary diarrhea
– skin: cystic fibrosis
Salt excess
– Sodium bicarbonate infusion
– i.v. saline, including flushes
– Malicious salt poisoning

(plasma sodium > 145 mmol/L) is caused by primary water deficit (with or without sodium loss) and commonly occurs because of insufficient water intake or impaired thirst mechanism (Table 121.3).

121.3.1 The Renin-Angiotensin-Aldosterone System

Aldosterone secretion is primarily regulated by the renin-angiotensin system and potassium (Fig. 121.3), and secondarily by ACTH. Hyponatremia, hypovolemia, low blood pressure and reduced renal perfusion stimulate the juxta-glomerular apparatus (cells surrounding the renal afferent arteriole, close to the macula densa) to secrete the proteolytic enzyme renin. Renin activates angiotensin I and II through angiotensinogen (liver) and angiotensin converting enzyme (ACE, lung), respectively. Angiotensin II has a potent vasopressor activity and, like potassium, acts directly on the glomerulosa cells in the adrenal cortex to stimulate aldosterone production. The distal tubule and collecting ducts of the kidney respond to increased aldosterone levels by increasing sodium reabsorption and excreting potassium and hydrogen. The mineralocorticoid receptor (MR) is responsive to both cortisol and aldosterone actions, but 11β-hydroxysteroid dehydrogenase (11β-HSD) activity at renal level prevents the conversion of cortisol to cortisone, which has very low mineralocorticoid receptor affinity. Aldosterone increases blood pressure by raising blood volume, and by increasing the sensitivity of arterial muscle to vasoconstrictors.

121.3.2 Antidiuretic Hormone

Antidiuretic hormone (ADH) or arginine vasopressin (AVP) is a nonapeptide that is synthesized as a large prohormone in the paraventricular and supra-optic nuclei of the hypothalamus. The most potent stimuli to ADH release are severe hy-

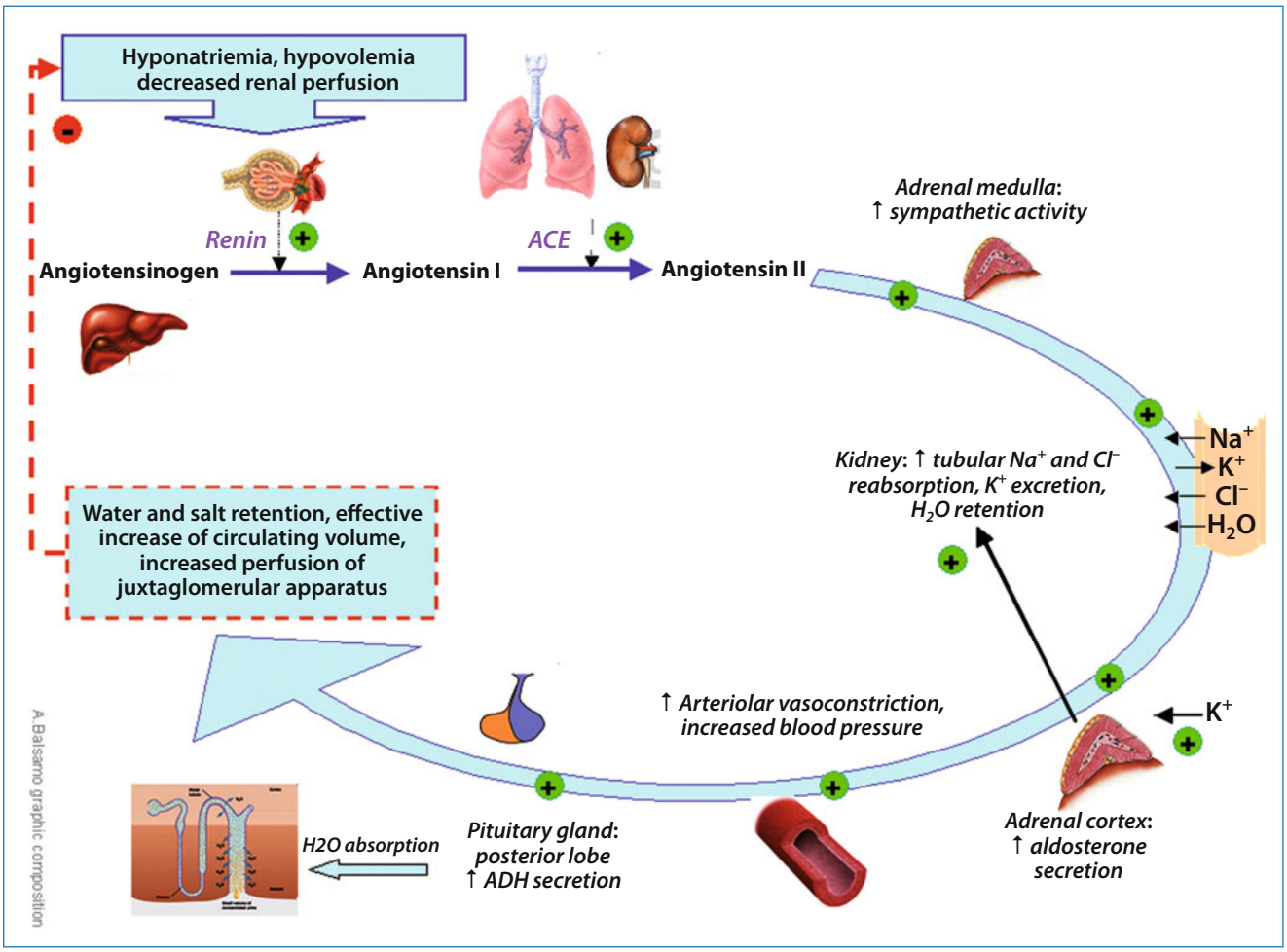


Fig. 121.3 Renin-angiotensin-aldosterone system

povolemia and increased plasma osmolality [16]. ADH acts via vascular V1 receptors and renal tubular V2 receptors. V1 receptor stimulation causes arterial vasoconstriction, and V2 stimulation increases renal free water re-absorption (due to the high interstitial osmolality). Relative or absolute deficiency of ADH causes DI. Central DI may be primary and is usually associated with other hypothalamo/pituitary gland developmental alterations and multiple hormone deficiencies. In ACTH deficiency, reduced cortisol levels may mask the effects of ADH deficiency, and fluid balance should be monitored at the start of hydrocortisone treatment for pituitary deficiency.

DI may rarely be caused by renal resistance to ADH (nephrogenic DI), an X-linked disease (Xq2.8), which is due to mutations in the arginine-vasopressin receptor 2 gene (*AVPR2*) [17].

In the newborn, ADH excess is more frequent than deficiency, and often due to underlying intracranial (e.g., cerebral hypoxic-ischemic) or lung (atrial natriuretic peptide) pathologies. Inappropriate ADH secretion is usually transient. The diagnosis is made by observing urine that is inappropriately concentrated in the presence of a low plasma osmolality. It is treated by fluid restriction.

121.3.3 Investigations

Attention to fluid balance is critical. This is achieved by daily or 12-hourly weighing with serial measurements of the acid-base status, blood concentrations of sodium, potassium, glucose, urea, creatinine, lactate and osmolality, and urine electrolytes and osmolality.

Urine and blood analysis for reducing substances may be indicated when there is a history of vomiting or jaundice and galactosemia is suspected.

In hyponatremic patients, measure plasma 17-OH-progesterone (steroid profile when available) and karyotype or FISH/PCR for Y fragments in case of genital ambiguity, for the diagnosis of CAH.

Ultrasound scans should be performed for the identification of kidney abnormalities (dysplasia, polycystic disease, renal vein thrombosis), adrenal hyperplasia or hemorrhage. Intracranial abnormalities (e.g., midline defects) in the presence of salt-water imbalance may indicate specific hormonal investigations.

121.3.4 Management

The goal for treating hyponatremia is normalization of the blood sodium concentration by identifying and treating the underlying disease. Patients with mild hyponatremia are almost always asymptomatic. Acute and severe hyponatremia requires prompt and careful administration and monitoring of serum sodium to avoid overcorrection and neurological damage. The long-term treatment depends on the diagnosis. If there is underlying renal disease, involvement by a pediatric nephrologist is indicated.

Management of hypernatremia involves fluid replacement, avoiding neurological complications due to rapid reduction of plasma sodium levels [18]. Fluid balance should be carefully monitored.

When DI is suspected, a vasopressin test may distinguish between central or nephrogenic DI [19]. This may be by continuous IV infusion of aqueous vasopressin with a starting dose of 1 mU/kg/h; range: 0.5–20 mU/kg/h) or DDAVP (IV or s.c. administration: start with 0.02 μg every 12 h and increase gradually until a suitable response is achieved) or by intranasal solution (start with 0.25 μg every 12 h, increasing gradually according to response; usual maximum dose 5-20 μg every 12 h).

121.4 Hypoglycemia and Hyperinsulinism

121.4.1 Hypoglycemia

The definition of the lower normal limit of blood glucose is controversial. A common definition of hypoglycemia is a plasma glucose level < 35 mg/dL (1.9 mmol/L) in term or < 40 mg/dL (2.2 mmol/L) in premature infants, although some studies have suggested 47–55 mg/dL (2.6–3.1 mmol/L). It is important to be aware that the blood glucose concentration usually decreases to 35 mg/dL (1.9 mmol/L) 1–2 hour after birth and then rises; about 15% of appropriate for gestational age (AGA) healthy full-term neonates have a blood glucose level < 50 mg/dL (2.8 mmol/L) during the first 72 hours of life [20–22]. Preterm and small for gestational age (SGA) newborn babies are unable to produce alternative fuels (ketone bodies) and are at increased risk of neuroglycopenia. In these babies, a blood glucose level above 55 mg/dL (3.1 mmol/L) is likely to be safer.

The overall incidence of hypoglycemia is about 1–5/1000 live births, although it can be as high as 15% in preterm infants and in those with intrauterine growth restriction (IUGR) or 37% in infants of diabetic mothers.

121.4.2 Classification and Causes

- Transient hypoglycemia: IUGR, SGA or large for gestational age (LGA) infants, premature or postmature neonates, infants of diabetic mothers (IDMs), intrapartum administration of glucose, perinatal stress, sepsis, birth asphyxia, hypothermia, polycythemia, hyperviscosity, shock, maternal drugs (terbutaline, propranolol, oral hypoglycemic agents) [23].

Table 121.4 Causes of hyperinsulinism

Condition	Inheritance	Features
Genetic forms		
Recessive K_{ATP} hyperinsulinism	AR	LGA at birth; unresponsive to diazoxide therapy
Focal K_{ATP} hyperinsulinism		Histologically appears as adenomatosis; LGA at birth, unresponsive to diazoxide therapy
Dominant K_{ATP} hyperinsulinism	AD	LGA at birth; responsive to diazoxide therapy; hypoglycaemia present at birth or later in infancy
GCK hyperinsulinism	AD	Responsive to diazoxide therapy; hypoglycaemia present at birth or later in infancy
GDH hyperinsulinism	AD	Persistent symptomatic hypoglycaemia and persistent asymptomatic hyperammonemia; responsive to diazoxide therapy
SCHAD hyperinsulinism	AD	Responsive to diazoxide therapy; severe hypoglycemia
Other		
Beckwith-Wiedemann syndrome	Various	LGA at birth; macroglossia, abdominal wall defects, hypoglycemia
Sotos syndrome		Macrocephaly, large hands and feet, hypertelorism, sometimes hypoglycemia
Pancreatic adenoma		
Oral sulfonylurea drugs		

AR autosomal recessive, *AD* autosomal dominant, *GDH* glutamate dehydrogenase, *SCHAD* short chain 3-hydroxyacyl CoA dehydrogenase.

- Persistent or recurrent hypoglycemia:
  - hyperinsulinism: see Table 121.4;
  - endocrine disorders: deficiency of GH, glucagon, cortisol, thyroid hormones, epinephrine;
  - inborn errors of carbohydrate metabolism: glycogen storage disease, glycogen synthase deficiency, galactosemia, fructose intolerance;
  - inborn errors of amino acid metabolism: maple syrup urine disease, propionic academia, methylmalonic academia, hereditary tyrosinemia, 3-hydroxy-3-methyl glutaric academia, glutaric academia type II;
  - inborn errors of fatty acid metabolism: defects of carnitine metabolism, acyl-coezyme dehydrogenase defects.

121.4.3 Clinical Presentation

Asymptomatic hypoglycemia is relatively common.

Symptomatic hypoglycemia may present with:

- Irritability, lethargy, abnormal cry, coma
- Sucking difficulty
- Tremors, seizures
- Pallor, cyanosis, sweating
- Hypothermia
- Hypotonia
- Apnea, irregular respiration
- Tachycardia.

121.4.4 Screening

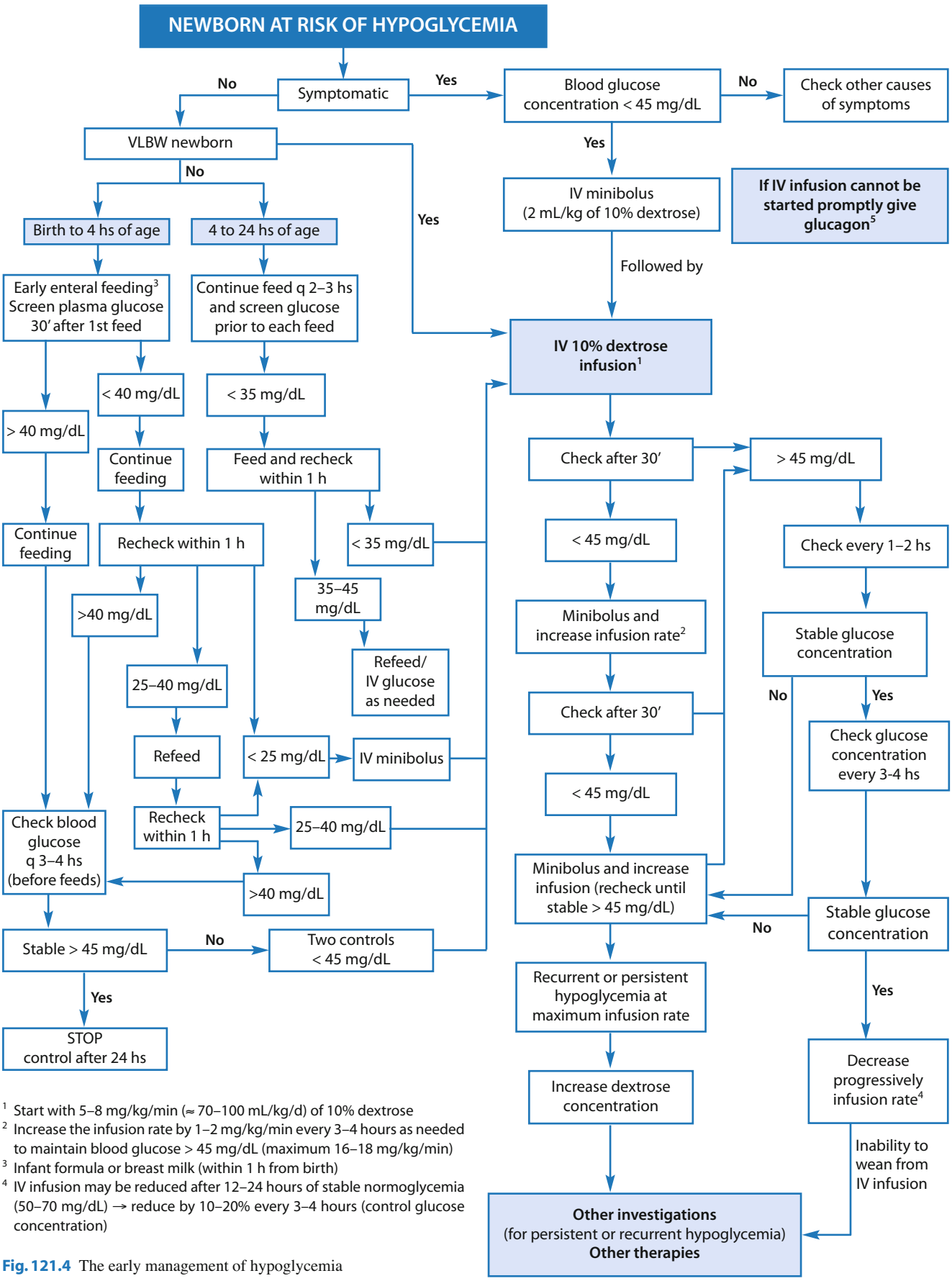
- High risk neonates
  - Birthweight > 4 kg or < 2 kg
  - SGA, LGA or intrauterine growth restriction
  - Infants of diabetic mothers (IDMs)
  - Preterm
  - Septic neonates
  - Clinical features of hypoglycemia.
- Mild to moderate risk neonates
  - Birth asphyxia, respiratory distress, low Apgar score
  - Neonates of mother treated with hypoglycemia-inducing drugs
  - Suspected inborn error of metabolism
  - Macroglossia or hemihypertrophy (Beckwith-Wiedeman syndrome).

121.4.5 Diagnosis

Semi-quantitative measurement of blood glucose levels should be used only as a screening test. To confirm the diagnosis, plasma or serum glucose levels should be measured in the laboratory by chemical analysis. Plasma glucose levels are about 10–18% higher than blood levels.

Other possible investigations (persistent or recurrent hypoglycemia): sample to be taken during hypoglycemia.

- First stage:
  - Insulin and C-peptide
  - Ultrasound imaging of pancreas
  - Ketones
  - Insulin/glucose (I/G) ratio. An I/G ratio > 0.30 suggests a hyperinsulinemic hypoglycemia. The diagnosis of hyperinsulinism is based on inappropriately high insulin levels.
- Second stage:
  - Cortisol, ACTH
  - GH
  - fT3, fT4, TSH
  - Glucagon
  - Free fatty acid
  - Amino acids (serum and urine)
  - Organic acids (urine)
  - Lactate, pyruvate, urate, acetoacetate
  - Ammonia
  - IGF-I, IGF-BPs
  - CT scan of the pancreas.



[1] Start with 5–8 mg/kg/min (≈ 70–100 mL/kg/d) of 10% dextrose
[2] Increase the infusion rate by 1–2 mg/kg/min every 3–4 hours as needed to maintain blood glucose > 45 mg/dL (maximum 16–18 mg/kg/min)
[3] Infant formula or breast milk (within 1 h from birth)
[4] IV infusion may be reduced after 12–24 hours of stable normoglycemia (50–70 mg/dL) → reduce by 10–20% every 3–4 hours (control glucose concentration)

Fig. 121.4 The early management of hypoglycemia

121.4.6 Management

For early management of hypoglycemia, see Fig. 121.4. The following points should also be considered:

- if postprandial glucose concentration is normal but the value before the next feed is low → IV therapy;
- IV glucose infusion should be the first line treatment in symptomatic hypoglycemia, and for newborns unable to tolerate enteral feeds, or with chronic or severe hypoglycemia (preterms, IUGR, infants of diabetic mothers, sepsis, suspected inborn errors of metabolism or endocrine disorders);
- persistent glucose infusion rate > 8 mg/kg/min suggests hyperinsulinism.

121.4.6.1 Persistent or Recurrent Hypoglycemia

In case of persistent or recurrent hypoglycemia, other treatments should be considered in association with IV glucose infusion:

- Corticosteroids: hydrocortisone (2.5 mg/kg over 3–5 min bid or tid).
- Diazoxide: PO 2–5 mg/kg tid; start with 5 mg/kg tid and reduce to the minimal dose effective. It is useful in hyperinsulinemic hypoglycemia. Concurrent administration of a diuretic (e.g., hydrochlorathiazide 3–5 mg/kg bid) is recommended → reduce fluid retetion due to diazoxide use and enhance diazoxide effects on blood sugar levels. Control blood pressure and glucose levels. If hypoglycemia is not responsive to 15 mg/kg/day, start octreotide infusion.
- Octreotide: starting dose 1 μg/kg qid IV or SC. Increase according to clinical effect to a maximum of 10 μg/kg qid. Some success in insulinomas and neonatal persistent hypoglycemia (nesidioblastosis). Recently the combination of octreotide with glucagon IV infusion (0.01–0.02 mg/kg/h → max 1 mg/24 h) has been reported for severe hypoglycemia secondary to hyperinsulinism. Monitor ECG and glucose levels during infusion.
- Growth hormone: 0.1–0.2 U/day SC; administer only when GH deficiency confirmed.
- Pancreatectomy: subtotal or total pancreatectomy following unsuccessful medical treatment of persistent neonatal hypoglycemia due to hyperinsulinism [24].

121.4.7 Beckwith-Wiedemann Syndrome

Beckwith-Wiedemann syndrome (BWS) is a rare congenital overgrowth disorder which occurs in about 1 out of every 13–17,000 live births.

Commonest clinical features in Table 121.5.

Table 121.5 Main clinical features of BWS

Clinical findings	Frequency
– Macrosomia (weight and length)	90%
– Hemihypertrophy (↑ risk for cancer)	10–15%
– Macroglossia	80%
– Cranio-facial anomalies (midface hypoplasia, a prominent occiput, and nevus flammeus)	60–70%
– Ear defects (creases or pit)	
– Anterior abdominal wall defects (omphalocele, umbilical hernia, diastasis recti abdominis)	75% 80%
– Hypoglycemia	50%
– Cardiac defects	25%
– Visceromegaly (liver, kidney, spleen)	
– Tumors (Wilms' tumor, hepatoblastoma, adrenal carcinoma, nephroblastoma)	7–20%

Table 121.6 Genetic alterations in BWS

Alterations detected on 11p15.5 region	Frequency*
– Duplication, translocation, inversion	1–2%
– Paternal uniparental disomy	10–20%
– Methylation abnormalities	55–65%
– CDKN1C mutations	5–10%

* In patients with "certain" clinical diagnosis of BWS.

121.4.7.1 Etiology

Only 15% of cases are familial due to complex inheritance. Chromosomal abnormalities in the highly imprinted region at chromosome 11p15.5 have been reported both in sporadic and familial cases (see Table 121.6) [25]. Several genes in the 11p region have been implicated, e.g. CDKN1C, IGF-2, and H19.

Diagnosis is based on clinical findings and on analysis of the 11p15 region.

121.4.7.2 Management and Prognosis

Glycemia should be monitored during the first days of life. Parents should be taught the clinical features of hypoglycemia to avoid hypoglycemia after discharge.

Blood alpha fetoprotein (AFP) concentration should be measured during the first years of life (hepatoblastoma).

Abdominal ultrasound scanning every 6 months to identify tumors. Repeated chest X-rays for the early diagnosis of thoracic neuroblastoma. Abdominal wall defects may require surgical treatment. Respiratory and feeding difficulties as well as speech abnormalities may be related to macroglossia and also require surgery.

Usually the prognosis is good, although an increased risk of cancers has been reported.

Developmental milestones are usually normal. Mental retardation is rare, provided that hypoglycemia is avoided [26].

121.5 Hyperglycemia

The definition of the upper normal limit of blood glucose in the newborn remains uncertain. A blood glucose level higher than 125 mg/dL (6.9 mmol/L) or plasma glucose level >150 mg/dL, regardless gestational age or postnatal age, are usually considered abnormal [27]. Nevertheless most neonatologists tolerate blood glucose levels of 150–180 mg/dL (8.3–11.1 mmol/L) in preterm infants. Plasma glucose levels are about 15% higher than blood levels because of the presence of erythrocyes which metabolise glucose.

Hyperglycemia is relatively common in extremely low birth weight infants (ELBW) and in SGA as a consequence of impaired glucose homeostasis. The incidence is inversely related to birth weight and gestation age. About 80% of neonates < 750 g are hyperglycemic during the first days of life. In full-term appropriately grown newborns (AGA), hyperglycemia is rare and mainly affects seriously ill neonates. Neonatal diabetes mellitus occurs in about 1:400.000 live births [29].

121.5.1 Causes

- Impaired glucose homeostasis: preterm and SGA infants.
- Iatrogenic hyperglycemia: excessive intravenous glucose infusion.
- Drugs: corticosteroids, caffeine, theophylline and phenytoin or maternal diazoxide.
- Stress: sepsis, intraventricular hemorrhage, birth asphyxia, painful procedures.
- Neonatal diabetes mellitus: transient or permanent.
- Absence of enteral feeding: diminished "incretin" secretion.

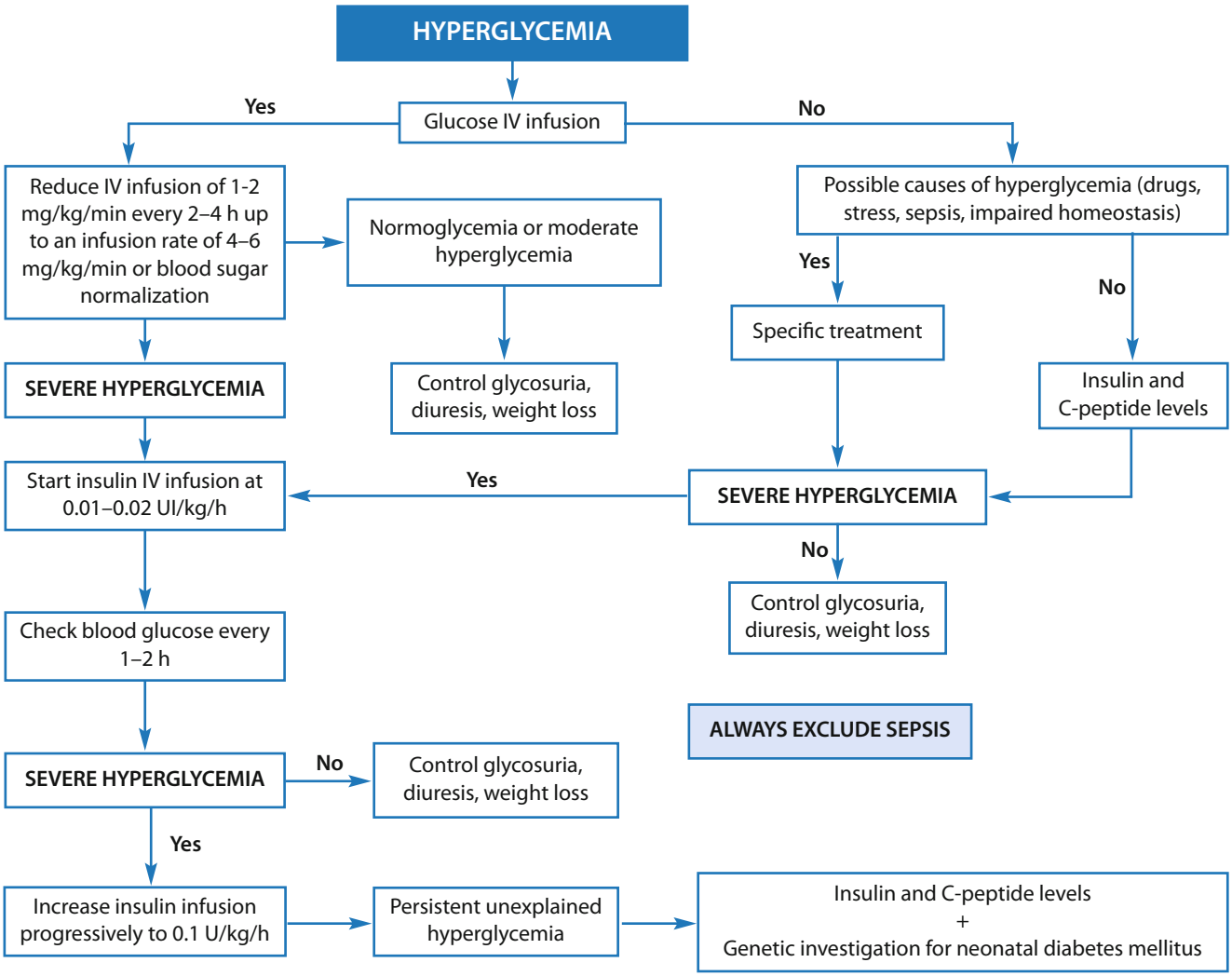


Fig. 121.5 The management of hyperglycemia. Based on evidence from [28]

121.5.2 Clinical Presentation and Diagnosis

Hyperglycemia may be associated with polyuria, dehydration, failure to thrive; rarely ketoacidosis. Semi-quantitative evaluation of blood glucose levels by reagent strips should only be carried out for screening. To confirm the diagnosis and before starting treatment, plasma or serum glucose concentrations should be measured by quantitative chemical analysis.

121.5.2.1 Other Investigations

- First stage:
  - acid/base status → diabetic ketoacidosis
  - serum osmolarity → each 18 mg/dL (1.0 mmol/L) rise in blood glucose causes an increase in serum osmolarity of 1 mOsm/L
  - urine glucose level → osmotic diuresis
  - blood examination → sepsis
- If persistent unexplained hyperglycemia:
  - blood insulin concentration
  - blood C-peptide concentration
  - genetic investigations for neonatal diabetes mellitus.

121.5.3 Complications

Hyperglycemia has been associated with high sepsis and mortality rates. There is an increased risk of intraventricular hemorrhage with high plasma osmolarity.

An osmotic diuresis increases the risk of dehydration and electrolytes imbalance.

121.5.4 Neonatal Diabetes Mellitus

Neonatal diabetes mellitus (NDM) is a rare monogenic metabolic disorder which presents as uncontrolled hyperglycemia during the first six months of life. The estimated incidence is about 1/300,000–500,000 live births. Two different clinical phenotypes have been recognized: transient neonatal diabetes mellitus (TNDM) and permanent diabetes mellitus (PNDM), which differ in the duration of insulin dependence. In TNDM, insulin treatment may be discontinued within 18 months after diagnosis although about 50% relapse during childhood or adulthood. Almost all neonates affected by NDM are SGA, although some with PNDM are not SGA [29].

121.5.4.1 Etiology

About 60–70% of patients affected by TNDM show abnormalities in the 6q24 region. TNDM is usually sporadic, although about one-third of cases have paternal transmission (paternal duplications, paternal isodisomy, methylation defect).

Most patients with PNDM carry mutations of the KATP channel (50–70%). Other genetic defects associated with PNDM are less common (Table 121.7).

121.5.4.2 Clinical Presentation

NDM presents as hyperglycemia, failure to thrive, dehydration and ketoacidosis during the first months of life. PNDM may occur as an isolated abnormality or as a part of a syndrome (Table 121.7). Patients with mutations of the ATP-sensitive potassium (KATP) channel (*KCNJ11* and *ABCC8* genes) may present with DEND syndrome (delayed development, epilepsy, NDM) or iDEND phenotype (DEND without epilepsy).

Table 121.7 Genes involved and clinical features of neonatal diabetes mellitus

Condition	Chromosome	Inheritance	Neonatal features
β-cell dysfunction			
ABCC8	11p15.1	AD	NDM +/– DEND
KCNJ11	11p15.1	AD	NDM +/– DEND o iDEND
Glucokinase	7p15-13	AR	PNDM
β-cell mass reduction			
FOXP3	Xp11.23-Xq13.3	X-linked	Exfoliative dermatitis, diarrhea, hemolitic anemia, thyreopathy, PNDM (IPEX syndrome)
INS	11p15	AD	PNDM
EIF2AK3	2p12	AR	PNDM, spondyloepiphysel dysplasia, hepatomegaly, renal failure, mental retardation (Wolcott-Rallison syndrome)
Abnormal islet development			
HNF1b	17cen-q21.3	AD	TNDM, renal abnormalities, genital malformation
PTF1 a	10p12.3	AR	PNDM, cerebellar hypoplasia
IPF1	13q21.1	AR	Pancreatic agenesis (PNDM and exocrine failure)
GLIS 3	9p	AR	PNDM, hypothyroidism

AR autosomal recessive, *AD* autosomal dominant.

121.5.4.3 Diagnosis

Persistent unexplained hyperglycemia during the first 6 months of life should be investigated for NDM. TNDM patients are usually younger and have lower insulin requirements. PNDM cannot be generally differentiated from TNDM on the basis of clinical features alone. Genetic investigations (chromosome 6, *KCNJ11*, *ABCC8*) allow the diagnosis for the majority of patients affected by NDM [30].

First line examination involves insulin and C-peptide. Second line examination involves genetic investigations.

121.5.4.4 Prognosis

Prognosis is related to the severity of the disease and to the rapidity of diagnosis and treatment. PNDM associated with other syndromes has a variable prognosis. Patients affected by TNDM should have long-term follow-up because of the risk of later relapse.

121.5.4.5 Treatment

Insulin therapy and high caloric intake are crucial for good metabolic control. We recommend the initial administration of intravenous insulin at 0.01–0.02 U/kg/h, adjusted according to blood glucose levels, followed by subcutaneous ultralente insulin at 0.4–0.6 U/kg/24 h.

When good metabolic control has been achieved, switching to oral sulfanylurea therapy may be attempted in patients affected by the *KCNJ11* and *ABCC8* gene mutations. The dose of glibenclamide may be as high as 0.8 mg/kg/day [30].

121.6 Hypocalcemia

Hypocalcemia is defined as a total serum calcium level < 2.1 mmol/L (8.5 mg/dL) or as an ionized calcium level < 1.1 mmoL (see also Chapter 49). Physiological hypocalcemia occurs after birth as a consequence of the end of transplacental calcium transfer, an insufficient supply through feeding and immaturity of the parathyroid glands with consequent reduced secretion of parathyroid hormone (PTH). The nadir in calcium levels occurs during the first 36–48 h of life.

Hypocalcemia is relatively common in neonates, affecting 30% of very low birth weight infants and 80–90% of infants born below 32 weeks [31, 32].

121.6.1 Clinical Presentation

Hypocalcemia is often asymptomatic. Clinical presentation includes non-specific symptoms and signs: signs of neuromuscular irritability (tremulousness, myoclonic jerks, exaggerated startle responses, seizures), lethargy, apnea, cyanosis, tachypnea, poor feeding, abdominal distension, vomiting, laryngospasm, tachycardia, prolonged QT interval on ECG, decreased myocardial contractility and heart failure.

121.6.2 Causes and Differential Diagnosis

The causes of neonatal hypocalcemia can be classified by time of onset (Table 121.8). The history should consider maternal, peripartum and postnatal events. The clinical examination

Table 121.8 Causes of hypocalcemia

Timing and causes	Mechanism
Early onset (within 72-96 h)	
– Preterm birth	Poor intake, increased calcitonin, decreased responsiveness to vit. D, hypoalbuminemia (decreased total but normal ionized calcium)
– Perinatal asphyxia	Increased calcitonin production and endogenous phosphate load, alkali therapy
– Infant of diabetic mother	Hypomagnesemia in the mother and the fetus leading to hypoparathyroidism in the infant
– Preeclampsia	
– Maternal hyperparathyroidism	Hypercalcemia with suppression of parathyroid activity in the fetus and the newborn (hypocalcemia may be prolonged)
Late onset (after 96 h)	
– Iatrogenic	Excessive intake of phosphate (phosphate therapy, feeding with phosphate-rich formula or cow's milk), citrated blood products, lipid infusion, bicarbonate therapy, loop diuretics and thiazide diuretics, glucocorticoids, *gentamicin use*
– Osteopenia of prematurity	Reduced calcium and phosphorus and increased ALP and PTH
– Parathyroid related disorders	See Table 121.9
– Vitamin D deficiency and resistance	Maternal deficiency of vitamin D often associated with use of anticonvulsants, inherited disorders of vitamin D metabolism (impaired 1-α-idroxilation-vitamin D dependent rickets type I and rarely 25-α-idroxilation), resistance to actions of vitamin D (vitamin D dependent rickets type II)
– Malabsorption of calcium or vitamin D	
– Renal failure	

Table 121.9 Parathyroid related disorders

Classification and mechanism	Diseases
Congenital or inherited parathyroid disorders	
– Aplasia or hypoplasia	DiGeorge syndrome, Velocardiofacial syndrome, HDR syndrome, Kenny-Caffey syndrome, Kearns-Sayre syndrome, X linked or autosomally inherited hypoparathyroidism, Vater association, Charge association, Gestational diabetes mellitus, fetal exposure to retinoic acid
– Impaired secretion of PTH	PTH gene mutations, activating mutations of the calcium sensing receptor
– Target organ resistance	Pseudohypoparathyroidism Type I (IA, IB, IC), Type II, Pseudo-pseudohypoparathyroidism
Non-congenital causes of parathyroid disorders	Hypomagnesemia and respiratory alkalosis

should look for dysmorphic features attributable to the 22q deletion syndrome (DiGeorge syndrome: cardiac defect, facial dysmorphisms, thymic aplasia, cleft palate). Other causes are hypoglycemia, hypomagnesemia, sepsis, meningitis, intracranial hemorrhage. Early onset hypocalcemia is usually self-limiting although hypocalcemia due to maternal hyperparathyroidism may be prolonged. Late onset hypocalcemia is often iatrogenic but vitamin D deficiency, renal failure and a parathyroid related disorders should be excluded (Tables 121.8 and 121.9).

121.6.3 Investigations

- Total and ionized calcium, phosphorus, magnesium, alkaline phosphatase
- pH, blood total protein, creatinine and electrolyte concentrations
- Blood parathyroid hormone, 25-OH vitamin D, 1,25-OH vitamin D concentrations
- Molecular genetic studies (if indicated)
- Urinary calcium, phosphorus, creatinine, cyclic adenosine monophosphate (cAMP) concentrations – measure calcium/creatinine ratio, tubular reabsorption of phosphate
- Maternal serum calcium and phosphorus concentrations.

Maternal hyperparathyroidism is characterized by high calcium, phosphorus and parathyroid hormone (PTH) levels, whereas, calcium and vitamin D levels are low in maternal vitamin D deficiency. Hypocalcemia associated with parathyroid dysfunction is distinguished by low serum calcium, increased serum phosphate, low or undetectable PTH levels. Inappropriately normal PTH levels may be present if some degree of PTH production is preserved. Serum $1,25(OH)_2D_3$ is low because PTH and hypophosphatemia are the main stimuli for the production of renal 25(OH)D 1α-hydroxylase. Elevated levels of PTH are present in syndromes associated with target organ resistance to PTH. Hypomagnesemia and respiratory alkalosis can cause transient abnormality of PTH secretion and target organ resistance to PTH.

Hypocalcemia secondary to vitamin D deficiency, inherited disorders of vitamin D metabolism or peripheral resistance to the action of vitamin D is differentiated by the presence of hypophosphatemia and elevated PTH levels.

In severe vitamin D deficiency or inherited disorders of vitamin D metabolism, the level of $1,25(OH)_2D_3$ is low. In moderate vitamin D deficiency, $1,25(OH)_2D_3$ levels may be normal or high due to the stimulation of the renal 1α-hydroxylase by PTH and hypophosphatemia. Levels of $1,25(OH)D_3$ may be high when there is peripheral resistance to the action of vitamin D (Table 121.8).

121.6.4 Congenital Parathyroid Disorders

Among some rare syndromes, hypoparathyroidism is associated with multiple malformations with various inheritance patterns (Table 121.9). Familial isolated hypoparathyroidism is caused by a mutation in the gene for the transcription factor *GCMB*, which is expressed in the PTH-secreting cells of the developing parathyroid [33]. An interstitial deletion/insertion at *Xq27.1* near *SOX3* gene is responsible for X-linked hypoparathyroidism [34]. Specific mutations in the parathyroid hormone gene have been found in families affected by congenital hypoparathyroidism [35].

Activating mutations in the calcium sensing receptor causes impaired PTH secretion and hypocalcemia with inappropriately normal PTH levels. There is clinical variability with cases affected by hypocalcemia and seizures and cases with asymptomatic hypocalcemia in the same family [36]. The term pseudohypoparathyroidism (PHP) refers to congenital forms of PTH resistance when hypocalcemia is associated with increased PTH levels and the administration of PTH fails to increase serum calcium levels and causes a phosphate diuresis. The first cases of PTH resistance were described by Albright. These patients were hypocalcemic and hyperphosphatemic and showed several features (rounded face, foreshortened fourth and other metacarpals, subcutaneous calcifications, short stature, obesity) that are now included in the syndrome, Albright's hereditary osteodystrophy (AHO). The classification of pseudohypoparathyroidism (see Appendix, p. 987) [37] is based on the variable presence of AHO and renal resistance to PTH.

121.6.5 Treatment

Oral treatment (3–4 mL/kg/day of calcium gluconate 10% in divided doses) should be given to babies with mild to moderate asymptomatic hypocalcemia or with mild neuromuscular irritability. Oral supplementation should be avoided for neonates at risk of necrotizing enterocolitis.

If there is symptomatic hypocalcemia, give IV 0.5–1 mL/kg of diluted solution of calcium gluconate 10% (1 mL = 9.3 mg of elemental calcium) slowly over 10 minutes (risk of bradycardia or cardiac asystole). The use of a central line is preferred. Maintenance IV treatment should be continued at 2–6 mL/kg/day.

The concomitant presence of hypomagnesaemia may lead to refractory hypocalcemia: give an initial dose of 100–200 mg/kg IV over 30 minutes (do not exceed 150 mg/minute) or as an IM injection and then continue with 20–50mg/kg/hour of diluted solution (usual dilution 4 grams magnesium sulphate to make 50 mL with 5% dextrose = 80 mg/mL; 0.25 mL × weight = 20 mg/kg/hour).

Hypoparathyroidism should be treated with alphacalcidiol 0.02–0.05 μg/kg/day.

If vitamin D deficiency is present, it should be treated first with oral supplementation: 5–50 μg/day (200–2000 U/day).

There is a single case report of recombinant PTH therapy in infants: safety and long-term effects are not known [38].

121.7 Hypercalcemia

During the neonatal period, hypercalcemia is less common than hypocalcemia. Hypercalcemia can be defined as ionized calcium (iCa) > 1.36 mmol/L (5.44 mg/dL) or total calcium (tCa) concentration > 2.75 mmol/L (11 mg/dL). tCa and iCa usually increase simultaneously but tCa is not predictive of iCa. Hypercalcemia may be due to iatrogenic causes (e.g., maternal intake of vitamin D), increased bone turnover or excessive intestinal or renal absorption.

121.7.1 Clinical Presentation

Mild hypercalcemia is often asymptomatic. Moderate to severe hypercalcemia usually presents with non-specific signs and symptoms: anorexia, vomiting, constipation, lethargy or irritability, hypotonia, seizures, coma, hypertension, polyuria and dehydration, bradycardia and shortening of the QT interval on the ECG. In its chronic form, the most common presentation is failure to thrive.

Physical examination is usually normal, except when there are syndromes or subcutaneous fat necrosis, Bony deformities may affect patients with severe hyperparathyroidism.

121.7.2 Causes

Neonatal hyperparathyroidism may become clinically manifest during the first 6 months of life. It can be primary or secondary to maternal pathological conditions (Table 121.10).

- Secondary hyperparathyroidism is due to maternal disease. Hypocalcemia, hypoparathyroidism or pseudohypoparathyroidism during pregnancy may lead to transient neonatal hyperparathyroidism. The prognosis is good and bone disease usually resolves within few months.
- Primary hyperparathyroidism
  - Familial hypocalciuric hypercalcemia is due to an inactivating mutation (autosomal dominant heterozygous) of the CaSR gene. Familial hypocalciuric hypercalcemia usually presents with an asymptomatic hypercalcemia, hypophosphatemia, mild hypermagnesemia, moderate hypocalciuria and inappropriately normal PTH. Hyperparathyroidism may resolves over several months.
  - Neonatal severe hyperparathyroidism is due to homozygous or double heterozygous mutations of the CaSR gene. Neonatal severe hyperparathyroidism presents with severe hypercalcemia, bone demineralization and hyperparathyroidism. Hypotonia, constipation and respiratory distress usually occur during the first days of life and may lead to death within a few weeks. Total parathyroidectomy and partial autotransplantation, and, more recently, pamidronate treatment may improve the outcome.

Williams syndrome is related to a microdeletion of chromosome 7 (q11.23). It is characterized by inconstant hypercalcemia, elfin facies, cardiovascular disease (80% have supravalvar aortic stenosis) and mental retardation.

Jansen's metaphyseal chondrodysplasia is attributable to a heterozygous mutation in the parathyroid hormone receptor (PTHR) which is situated in the kidney, bone, and growth plate. At birth, these children may have either a normal phenotype or micrognathia, prominent eyes, hypertelorism and progressive short-limbed dwarfism.

Blue diaper syndrome is a familial disease resulting from a defect in the intestinal transport of tryptophan. Hypercalcemia and nephrocalcinosis are usually manifest a few months after birth. The blue color of the urine is due to a tryptophan metabolite. The clinical presentation is with failure to thrive, gastrointestinal disturbances, recurrent fever and irritability.

Idiopathic infantile hypercalcemia is often considered part of Williams syndrome. The clinical phenotype is variable but symptomatic hypercalcemia generally resolves during childhood. Hypercalcemia is secondary to increased intestinal calcium absorption, although the pathogenesis and inheritance are not clear.

Subcutaneous fat necrosis (SFN) may induce hypercalcemia probably due to excessive intestinal calcium uptake because of increased $1,25(OH)_2D_3$ production in the lesion. SFN

Table 121.10 Causes of hypercalcemia

Causes	Description
Iatrogenic	Excessive intake of calcium or vitamin A (increased bone resorption) or vitamin D (increased intestinal uptake); thiazide diuretics (reduced renal clearance); phosphate deficiency (especially in VLBW infants with low phosphate in parenteral nutrition)
Parathyroid related	Secondary hyperparathyroidism: maternal hypocalcemia, hypoparathyroidism or pseudohypoparathyroidism Primary hyperparathyroidism: familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism
Syndromic form	Williams syndrome, Jansen's metaphyseal chondrodysplasia, Blue diaper syndrome, idiopathic infantile hypercalcemia; IMAGe syndrome (IUGR, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital anomalies)
Other form	
– Subcutaneous fat necrosis	Excessive intestinal calcium uptake due to increase $1,25(OH)_2D_3$ production from macrophages infiltrating the lesion
– Distal renal tubular acidosis	Acidosis enhance calcium bone resorption; reduced renal excretion is secondary to reduced glomerular filtration rate
– Severe infantile hypophosphatasia	Severe hypercalcemia and bone deformity; low ALP
– Congenital endocrine diseases (congenital hypothyroidism, adrenal insufficiency)	Unknown mechanism
– Metabolic disorders	Congenital carbohydrate malabsorption (disaccharidase deficiency, congenital lactase, glucose-galactose or sucrase-isomaltase deficiency), glycogen storage disease type 1a
– Tumor-related hypercalcemia	Increased production of PTH related protein (hepatic sarcoma, renal adenoma, rhabdoid tumors…)

usually affects large for gestational age term newborns with birth trauma. There have been reports of SFN following hypothermia and birth asphyxia. It presents as indurated plaques or nodules. Hypercalcemia is rarely associated with SFN, but is a severe complication which may lead to death if not promptly treated [39].

Severe infantile hypophosphatasia is an autosomal recessive disorder (chromosome 1) associated with extremely low serum and tissue alkaline phosphatase. These patients have severe bone demineralization. The clinical presentation ranges from a lethal form that presents soon after birth to a milder form that presents later. Laboratory findings include hypercalcemia, low serum alkaline phosphatase, and high urinary pyrophosphate and phosphoethanolamine concentrations.

Tumor related hypercalcemia (paraneoplastic syndrome) is secondary to an elevated level of PTHrP that lead to osteoclast rebsorption of bone, renal resorbtion of calcium, renal loss of phosphate.

121.7.3 Diagnostic Evaluation

A blood ionized calcium concentration > 1.36 mmol/L (5.44 mg/dL) and/or total serum calcium concentration > 2.75 mmol/L (11 mg/dL).

In the presence of acidemia or hypoalbuminemia, total serum calcium concentration may be normal in the presence of high ionized calcium levels.

121.7.4 Other Investigations

Calcium, phosphorus, vitamin A and vitamin D intake of the mother and baby. Birth trauma (subcutaneous fat necrosis), drugs during pregnancy and inherited disease should be considered.

- First stage
  - Electrolytes: total and ionized blood calcium, phosphorus and magnesium concentrations
  - Blood: pH, total protein and albumin, creatinine, alkaline phosphatase concentrations
  - Urine: calcium, phosphorus, creatinine concentrations
  - Other investigations: ECG, chest and limb X-rays, renal ultrasound
- Second stage
  - Parathyroid hormone, 25-OH vitamin D, 1,25-OH vitamin D, parathyroid hormone-related protein (PTHrP)
  - Molecular genetic studies

121.7.5 Management

Asymptomatic mild hypercalcemia should be managed conservatively. Hypercalcemia following maternal hypocalcemia is reduced by appropriate intake of Ca/P.

Moderate to severe hypercalcemia requires prompt intervention:

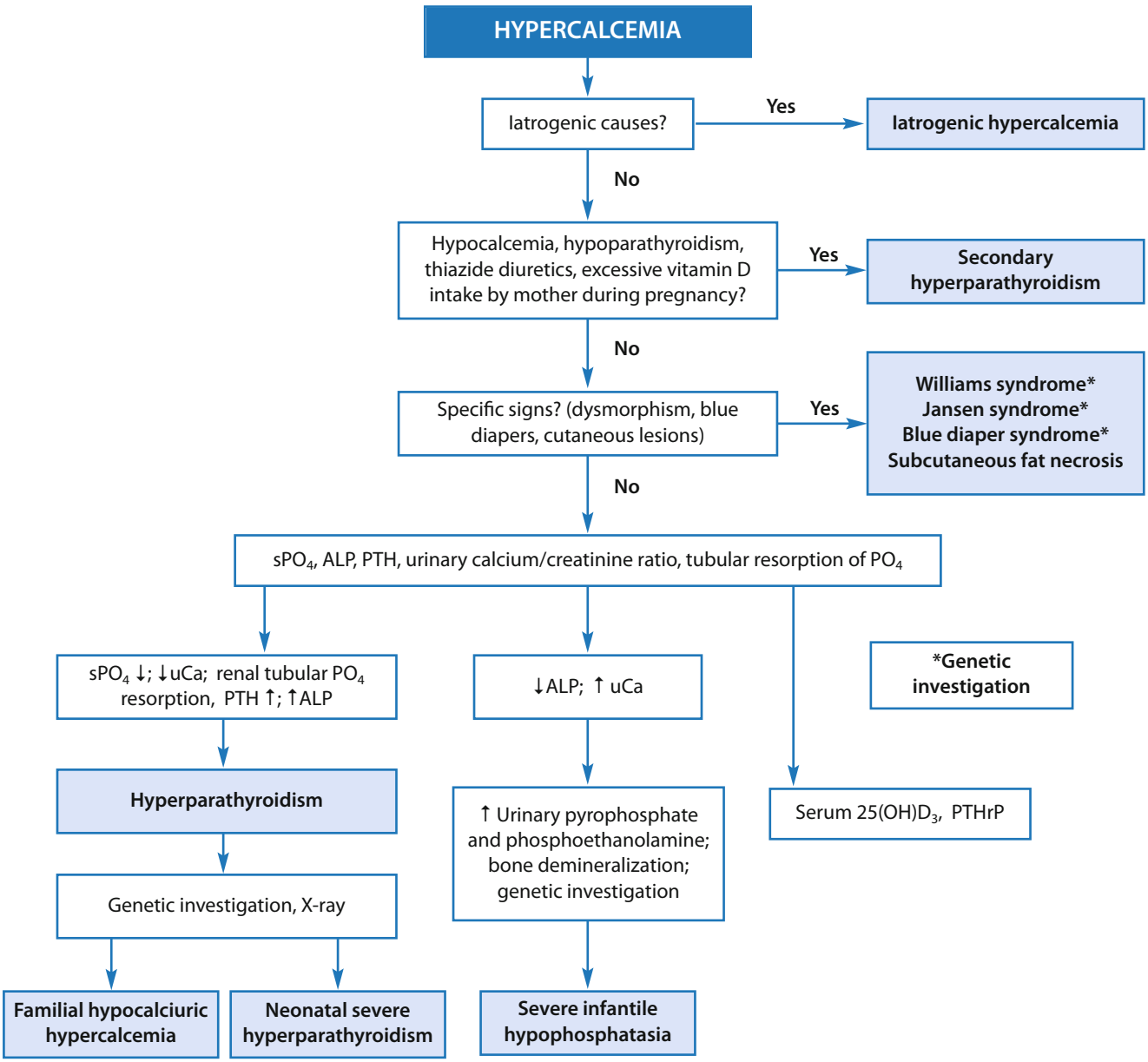


Fig. 129.6 Differential diagnosis of hypercalcemia

- Limit Ca and vitamin D intake (suspend oral/parenteral supplementation)
- Hyperhydration with normal saline (about twice maintenance requirements)
- Loop diuretics (furosemide 2 mg/kg) to increase calciuria → avoid dehydration and electrolyte imbalance

121.7.5.1 Other Specific Interventions

- Prednisone (2 mg/kg) → short course may decrease intestinal calcium absorption and bone resorption and increase renal excretion; especially useful in vitamin D excess (avoid long-term use → side effects)
- Calcitonin (4–8 IU/kg IV or IM every 12 hours) → reduces serum calcium concentration but the effect is short lasting.
- Bisphosphonates such as pamidronate (0.5–2.0 mg/kg) are used in the treatment of subcutaneous fat necrosis and may delay parathyroidectomy in neonatal severe hyperparathyroidism (NSHPT) → risk of severe hypocalcemia.
- Total parathyroidectomy and partial autotransplantation may be necessary in the severe form of NSHPT [40].

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Appendix "Rare diseases" with associated hormone abnormalities. (*On behalf of A. Balsamo, P. Ghirri, A. Cicognani and A. Boldrini*)

Disease/OMIM	Gene/OMIM/ map gene locus	Clinical and diagnostic features	Possible specific treatment
Aarskog-Scott S./ 305400 and 100050	*FGD1*/300546 Xp11.21	Aarskog syndrome affects mainly males, but females may have a milder form. *Short stature*, hypertelorism, and *shawl scrotum* are the main clinical features. Facial dysmorphisms may include rounded face, widow's peak hairline, hypertelorism, blepharoptosis, downslanting eye slits, small nose with anteverted nostrils, broad philtrum, and ear anomalies. Other signs described:" simian crease" in the palm of hand, clinodactyly of the 5th finger, brachydactyly, *delayed sexual maturation*, *criptorchidism*, pectus excavatum. Mild to moderate mental problems are usually present.	–
Antley-Bixler S./207410	*FGFR2*/176943 10q26	There is evidence that the ABS can be caused by mutation in a fibroblast growth factor receptor gene (FGFR2) and is characterised by craniosynostosis (trapezoidocephaly, midface hypoplasia, proptosis, choanal stenosis or hypoplasia), humeroradial synostosis, bowing of the femora and ulnas, long bone fractures, long slender fingers with camptodactyly, cardiac and renal malformations.	–
	POR/124015 7q11.2	ABS-like phenotype with *ambiguous genitalia and disordered steroidogenesis*, is a distinct disorder caused by mutations in the cytochrome P450 oxidoreductase (POR) gene (see Chapter 123). *Steroid profile between 21-OHase and 17-OHase deficiency*.	Hydrocortisone
Beckwith-Wiedemann S./ 130650	*CDKN1C*/600856 *H19*/103280 *IGF2*/147470 11p15.5, 11p15.5, 11p15.5, 5q35	See § 121.4.7	See § 121.4.7
CHARGE association/214800	*SEMA3E*/608166 *CHD7*/608892 8q12.1 7q21.11	Patients affected presents with a various association of: Coloboma, Heart defects, choanal Atresia, Renal and *Genital anomalies*, and Ears abnormalities. Frequently reported features include: facial palsy, cleft palate, dysphagia and *growth retardation*. May be associated with *hypocalcemia*.	Support theraphy, correction of choanal atresia and heart . defects
Denis Drash S./194080	*WT1*/607102 11p13	*Genital ambiguity*, congenital nephropathy and Wilms' tumor. *Proteinuria*, *renal impairment*. Frasier syndrome and Meacham syndrome are allelic disorders with similar clinical features.	Surgery for Wilms' T.
Frasier S./136680	*WT1*/607102 11p13	*Genital ambiguity*, *streak gonads*, and XY karyotype; frequently develop gonadoblastoma; progressive glomerulopathy; Wilms' tumor is not a usual feature. Often the diagnosis is during adolescence or adulthood. No mutant protein is produced by the mutations in WT1 causing Frasier syndrome. Instead, the mutation results in an altered ratio of the 2 splice isoforms of the protein, those with and those without the extra 3 amino acids (KTS).	Prophylactic bilateral gonadectomy; dialysis and renal transplantation
Meacham S./608978	*WT1*/607102 11p13	*46,XY karyotype, with complex sex reversal or ambiguous genitalia* (retention of müllerian annexes, double vagina), and congenital diaphragmatic hernia. Other symptoms, including heart, pulmonary, and genital defects, are variable. All patients die early in life. None has renal mesangial sclerosis or Wilms' tumor. Heterozygous mutations in the WT1 gene were identified in some cases.	Surgical correction of malformations
DiGeorge S. (***DGS***)/188400	*TBX1*/602054 22q11.2	A deletion on Cr 22 alters the migration of *neural crest*-derived tissues, affecting development of the pharyngeal pouches and arches. The main clinical features of Digeorge syndrome are cardiac abnormality (mainly tetralogy of Fallot, interrupted aortic arch, ventricular septal defect, and persistent truncus arteriosus), Abnormal facies (hypertelorism), T cell deficit secondary to thymic hypoplasia, Cleft palate and Hypocalcemia due to *hypoparathyroidism*. The acronym "CATCH phenotype" has been used to describe this association. Recurrent infections are due to T-cell deficit; seizures secondary to *hypocalcemia* are common.	Cardiac surgery, antibiotics and Vitamin D and calcium supplementation

		Renal anomalies, hearing loss, skeletal anomalies, autoimmune diseases, feeding difficulties, and developmental delay have been frequently reported. DGS is overlapped with other 2 syndromes: conotruncal anomalies face (CTAF) and velocardiofacial syndrome (VCFS)	
Dubowitz S./223370	? / – AR inheritance	Developmental disorder involving IUGR, short stature, microcephaly, mild mental retardation. Behavior problems, eczema, and unusual and distinctive facies are constant features. Various minor malformations, such as pilonidal dimples, submucous clefts, hypospadias, cryptorchidism, ptosis, high-pitched voice and sparse hair, were also seen. *Persistent low serum lipid levels and an arachnoid cyst have been recently associated*.	–
EEC1 S./129900	? / – 7q11.2-q21.3	Ectrodactyly, Ectodermal dysplasia with severe keratitis and Cleft lip palate (EEC type 1); EEC1 can be associated with *GH deficiency and/or genital anomalies* secondary to developmental defects of the hypothalamus. Another form of the disorder, designated EEC3 is caused by mutation in the *TP63* gene, but without apparent endocrine disorders.	GH treatment
Fanconi Anaemia/227650	*FANCA*/607139 16q24.3	FA-A is the most common complementation group, accounting for approximately 65% of all affected individuals. All marrow elements are usually affected, resulting in anemia, leukopenia, and thrombocytopenia (may not manifest in the neonatal period). Pigmentary changes in the skin (cafè-au-lait spots) and malformations of the heart, kidney, and limbs (aplasia of the radius, thumb deformity) are associated congenital features. *Abnormal sexual development* (especially in males) may be present and represents *hypergonadotropic hypogonadism*. *The gold-standard screening test for FA is based on the characteristic hypersensitivity of FA cells to the crosslinking agents, such as mitomicin C or diepoxybutane.This is usually a reliable technique to identify FA homozygotes but could not be able to identify individual FA heterozygotes*.	Stem cell transplantation
Fanconi-Bickel S./227810	*SLC2A2*, *GLUT2*/ 138160 3q26.1-q26.3	Is a metabolic syndrome secondary to impaired utilization of glucose and galactose. Glycogen is accumulated in the liver and in the kidney. The proximal renal tubular dysfunction leads to polyuria and dehydration. The impaired utilization of glucose and galactose induces fasting hypoglycemia and post-prandial hyperglycemia and hypergalactosemia. *Growth is compromised* and rickets and osteoporosis later in life were constant	Diet (low galactose intake, vitamin D, phosphate, water and electrolytes supplementation)
IMAGe association/300290	*DAX1*/300473 Other genes	See § 121.7	–
Kallmann S.: ***KS1*** 308700/ ***KS2*** 147950/***KS3*** 244200/ ***KS4*** 610628/***KS5*** 612370/ ***KS6*** 600483	*KAL1*/308700 Xp22.3 *FGFR1*/136350 8p11.2-p11.1 *PROKR2*/607123 20p13 *PROK2*/607002 3p21.1 *CHD7*/608892 8q12.1 *FGF8*/600483 10q24	KS1: hypogonadotropic hypogonadism and anosmia (constantly); sometimes: midline cranial anomalies (choanal atresia and cleft lip or palate), optic nerve atrophy, deafness, cryptorchidism, renal agenesis. Loss-of-function mutations in KAL1 and FGFR1 account for approximately 20% of all cases of Kallmann syndrome and mutations in the PROKR2 and PROK2 genes account for an additional 10%. No postnatal increase of LH and testosterone; blunted response to GnRH and hCG tests.	Since therapeutic success with substitution therapy is probably age dependent, early diagnosis is important.
Kearns-Sayre S./530000	Various mitochondrial deletions	The main features reported for KSS are: ophthalmoplegia, pigmentary degeneration of the retina, and cardiomyopathy. Other signs and symptoms are: muscle weakness, ptosis, cns dysfunction (cerebellar ataxia and mental retardation), cataracts, cardiac involvemement (bradycardia or congestive cardiac failure), endocrine dysfunction (hypogonadism, *diabetes mellitus*, *growth hormone deficiency* and *hypoparathyroidism*).	Coenzyme Q10 administration and vitamin supplements

		Diagnosis is confirmed with muscle biopsy of the orbicularis muscle. Blood lactate and pyruvate levels are usually elevated. CSF analysis shows elevated proteins (>100 mg/dL) and lactate levels.	
Kenny-Caffey S. type 1/244460 ***Kenny-Caffey S. type 2***/127000	*TBCE*/604934 1q42-q43	KCS is a skeletal disorder characterized by growth retardation, craniofacial anomalies and small hands and feet. Bone X-rays show cortical thickening of long bones with medullary stenosis and absent diploic space in the skull. Hypocalcemia consequent to *hypoparathyroidism* may lead to convulsions. *Blood examinations reveal low levels of calcium, phosphorus and PTH*. In type 2 hypocalcemia may be transient.	Prevent severe hypocalcemia
Laron S./262500 5p13-p12	*GHR*/600946	A GH receptor mutation lead to a *GH insensitivity*. Neonatal birthweight and leght are usually normally. Postnatal growth is severely compromised. Developmental milestones are delayed and mental retardation may be present. Facial dysmorphism may be present at birth (frontal bossing, hypoplastic nasal bridge, shallows orbits, blue sclera, decreased vertical dimension face). Muscoloskeletal involvement include hip dysplasia, hypotrophic musculature and osteopenia. Obesity is usually present. Sexual development is impaired with *micropenis in childhood* but normal genital growth during adolescence. The puberty is delayed but the reproductive function is conserved. *Blood investigations may reveal hypoglycemia and hyercolesterolemia. IGF-I levels are low despite serum levels of GH normal or elevated. X-ray investigations show bone age delay*.	Treatment with synthetic IGF1
Laurence-Moon S./245800 & ***Bardet-Biedl S.***/209900	*BBS1-14*/ 209901-606151-608845-600374-603650-604896-607590-608132-607968-610148-602290-610683-609883-610142 11q13/;16q21; 3p12-q13; 15q22.3; 2q31; 20p12; 4q27; 14q32.11; 7p14; 12q; 9q33.1; 4q27; 17q23; 12q21.3	BBS is a genetically heterogeneous disorder. Polydactyly, *obesity*, retinitis pigmentosa, renal anomalies, mental retardation with progressive ataxia and spastic diplegia. These patients have *hypogonadism*. There has been longstanding uncertainty as to the relationship between the Laurence-Moon syndrome and the Bardet-Biedl syndrome.	Support therapy (physiotherapy, rehabilitation,…)
Majewski S./263520	–	Malformations included median cleft lip, pre- and postaxial polysyndactyly, short ribs and limbs (tipically disproportionate shortening of the tibia), *ambiguous genitalia* and anomalies of epiglottis, kidney, CNS and lung.	–
Mitochondrial trifunctional protein deficiency/609015	*HADHA*/600890 *HADHB*/143450 2p23, 2p23	Trifunctional protein deficiency lead to a decreased activity of 3 enzymes. Clinically it may presents with SIDS, a Reye-like syndrome, cardiomyopathy, and/or skeletal myopathy. Early onset symptoms are *hypotonia*, feeding difficulties, *lethargy* and hypoketotic *hypoglycemia*. Liver, *heart*, and respiratory system may be involved and may lead to sudden death. The late-onset form is usually milder.	Dietary treatment
Müllerian duct aplasia, unilateral renal agenesis, and cervicothoracic somite anomalies (MURCS)/601076	–	MURCS is probably the second most frequent cause of *primary amenorrhoea* after Turner syndrome. It is an association of: müllerian duct aplasia, Unilateral Renal aplasia, and Cervicothoracic Somite dysplasia. Other features reported in literature: *absent vagina*, Klippel-Feil deformity of the cervical spine, *short stature*, and conductive deafness.	–
Noonan S.: ***NS1*** 163950/ ***NS2*** 605275/***NFNS*** 601321/ ***NS3*** 609942/***NS4*** 610733/ ***NS5*** 611553 /	*PTPN11*/176876 *NF1*/162200 *KRAS*/190070 *SOS1*/182530 *RAF1*/164760 12q24.1, 17q11.2, 12p12.1, 2p22-p21, 3p25	The estimated incidence is of 1 in 1,000 to 2,500 live births. Mutations in the *PTPN11* gene accounted for about half the patients reported (NS1). Mutations in the *NF1* (site of mutations causing classic neurofibromatosis type I) have been found in neurofibromatosis-Noonan syndrome (NFNS). De novo germline mutations of the KRAS gene account for less than 5% of NS3 cases. Other forms of Noonan syndrome, such as NS2, NS4 and NS5 have been identified.	GH treatment may be usefull in some cases

		The syndrome is characterized by hypertelorism, a downward eyeslant, and low-set posteriorly rotated ears Other features include *short stature*, a short neck with webbing or redundancy of skin, cardiac anomalies, epicanthic folds, deafness, motor delay, and a bleeding diathesis. *Metacarpophalangeal pattern profile (MCPP) analysis may be useful as a diagnostic tool in screening subjects for Noonan syndrome (PubMed ID: 10797437).*	
Pallister-Hall S./146510	*GLI3*/165240 7p13	Hypothalamic hamartoblastoma (may be lethal in neonatal period), postaxial polydactyly, and imperforate anus. Sometimes had laryngeal cleft, abnormal lung lobation, renal agenesis or dysplasia, short 4th metacarpals, nail dysplasia, multiple buccal frenula, *hypoadrenalism*, *genital anomalies (microphallus or hypospadia)*, congenital heart defect, and *IUGR*.	–
Pendred S./274600	*SLC26A4*/605646 7q21	PDS is the most common syndromal form of deafness; typical features are: bilateral sensorineural hearing loss and *goitre* (usually associated with compensated hypothyroidism) with occasional overt *hypothyroidism*. Mental retardation has been reported.	*Cochlear implants*, speech support and sometimes thyroxine
Permanent and transient neonatal diabetes mellitus/ 606176/601410/610374/ 610582/610199/609069/ 304790/226980/137920/ 260370	*ABCC8*/600509 *KCNJ11*/600937 *GCK*/138079 *ZFP57*/612192 *PLAGL1*/603044 *ABCC8*/600509 *GLIS3*/610192 *PTF1A*/607194 *FOXP3*/300292 *EIF2AK3*/604032 *HNF1B*/189907 *IPF1*/600733 See § 121.5.4 for chromosome mapping	See § 121.5	See § 121.5
Prader-Willi S./176270	*NDN*/602117 *D15S227E*/600161 *D15S226E*/600161 *SNRPN*/182279 15q11-q13	Muscular hypotonia, respiratory distress and feeding difficulties may be present at birth. Later in life: *obesity* and hyperphagia, mental retardation, *short stature*, *hypogonadotropic hypogonadism*, and small hands and feet. It can be considered an autosomal dominant disorder caused in 75% of the patients by deletion of the 15q11-q13 and in 24% by maternal uniparental disomy of the proximal arm of chromosome 15.	GH treatment may improve growth management
Robinow S./AD 180700; AR 268310	? / – *ROR2*/602337 9q22	Is a genetically heterogeneous disorder characterized by short-limbed *dwarfism*, abnormal face (fetal face with short, upturned nose, prominent forehead and flat nasal bridge) and *external genitalia* (micropenis, hypospadia, reduced size clitoris and underdeveloped labia minora). Adult fertility in females seems to be normal which may explain a lack of male to male transmission in the AD form. The AR form tends to be more severe.	–
Sanjad-Sakati S. or ***Hypoparathyroidism-retardation-dysmorphism S. (HRD)***/241410	*TBCE*/604934 1q42-q43	HRD is a rare form of congenital *hypoparathyroidism* associated with growth and mental retardation and seizures. Facial dysmorphism are typical: deep-set eyes, abnormal external ears, depressed nasal bridge with beaked nose, long philtrum, thin upper lip, micrognathia. *Hypocalcemia is associated with hyperphosphatemia and very low concentrations of PTH.*	–
Septo-optic Dysplasia/182230	*HESX1*/601802 3p21.2-p21.1	Clinically heterogeneous disorder defined by any combination of optic nerve hypoplasia, pituitary gland hypoplasia, and midline abnormalities of the brain, including absence of the corpus callosum and septum pellucidum. See § 121.1.	Hormone deficient substitutive treatment
Silver-Russel S./180860	*H19*/103280 and *IGF2*/147470 11p15.5, 7p11.2	SRS is caused by the epigenetic changes of DNA hypomethylation at the telomeric imprinting control region (ICR1). SRS can also be caused by maternal uniparental disomy of chromosome 7. Severe IUGR, poor postnatal growth, craniofacial features such as a triangular shaped face and a broad forehead, body asymmetry, and a variety of minor malformations.	GH treatment; GnRH analogues

Smith-Lemli-Opitz S./270400	*DHCR7*/602858	See Chapter 123	–
Sotos S./117550	*NSD1*/606681 5q35	Also known as cerebral gigantism, is characterized by excessive growth during the first 2-3 years, *mental retardation*, delayed developmental milestones and *hypotonia*. Birth length is increased (90-97th centiles), as well as skull dimension. Facial dysmorphism included protrusive forehead, *hypertelorism*, downslanting eyes, highly arched palate, prognathism, pointed chin, large ears and pointed chin. *Scoliosis*, seizures, heart and kidney defects, hearing loss, and problems with vision. Are less common features. An increased risk of developing tumors is associates with Sotos syndrome. *X-ray investigations show an advanced bone age*.	
Urioste S./235255 (müllerian derivatives, persistence of, with lymphangiectasia and postaxial polydactyly)	? / – AR or X linked	*Genital ambiguity* (46,XY with müllerian duct remnants), IUGR, lung anomalies, redundant nuchal skin, postaxial polydactyly and lymphangiectasia are constant. Hepatic failure and hypoproteinemia are frequent.	Usually early death
VATER association/192350	*PTEN*/601728 10q23.31	The acronym means: Vertebral defects, Anal atresia, Tracheaesophageal fistula associated with Esophageal atresia, Radial and Renal abnormalities. VACTERL association includes Cardiac malformations and Limb anomalies. May be associated with *hypocalcemia*.	Surgical correction of malformation
Very long-chain acyl-coenzyme A dehydrogenase deficiency (VLCAD)/201475 ***Long-chain acyl-coenzyme A dehydrogenase deficiency (LCAD)***/201460 ***Medium-chain acyl-coenzyme A dehydrogenase deficiency (ACADM)***/201450	*ACADVL*/609575 17p13 *ACADL*/609576 2q34-q35 *ACADM*/607008 1p31	VLCAD: usually presents with nonketotic *hypoglycemia*, *lethargy* and *muscle* weakness. High prevalence of liver dysfunction and life-threatening events (heart disease) have been reported. *Diagnosis is confirmed by VLCAD protein in skeletal muscle biopsies*. LCAD: nonketotic hypoglycemia, hepatomegaly, cardiomegaly, and hypotonia are the main clinical features. *Total plasma carnitine concentration is low. As in ACADM dicarboxylic acids in the urine are relatively low*. ACADM: may presents with intolerance to prolonged fasting and recurrent episodes of hypoglycemic coma. *Laboratory findings include medium-chain dicarboxylic aciduria, impaired ketogenesis, and low plasma and tissue carnitine levels*.	
Vitamin D-dependent rickets, type I (VDDRI)/264700 ***Vitamin D-dependent rickets type II (VDDRII)***/277440	*CYP27B1*/609506 12q13.1-q13.3 *VDR*/601769 12q12-q14	VDDR I is an hereditary selective enzymatic deficiency that lead to lack of the active form of vitamin D (1,25-dihydroxyvitamin D3). Usually presents with defective bone mineralization and clinical features of rickets (hypotonia, tetany, hypocalcemic seizures, irritability, motor retardation, deformations, and growth failure). *Laboratory findings include hypocalcemia, increased phosphaturia, markedly decreased serum 1,25-dihydroxyvitamin D3, normal serum 25-hydroxyvitamin D3, aminoaciduria, hyperparathyroidism, and absence of 1-α-hydroxylase activity*. VDDR II is caused by autosomal recessive mutations of the vitamin D receptor. Most patients have total alopecia in addition to rickets. Patients display rapidly progressing rachitic bone changes, hypocalcemia and secondary hyperparathyroidism. *Serum levels of 1,25(OH)$_2$D$_3$ are very high*.	VDDR I: large doses of vitamin D2 and physiologic doses of 1-α-hydroxyvitamin D$_3$ VDDR II: limited success to high doses of oral calcium and supraphysiologic doses of 1,25(OH)$_2$D$_3$ (1058)
Hypophosphatemic rickets X-linked dominant (XLH)/307800 ***Hypophosphatemic rickets autosomal dominant (ADHR)***/193100 ***Hypophosphatemic rickets autosomal recessive (ARHR)***/241520 ***Hypophosphatemic rickets X-linked recessive***/300554 ***Hypophosphatemic rickets with hypercalciuria***/241530	*PHEX*/300550 Xp22.2-p22.1 *FGF23*/605380 12p13 *DMP1*/600980 4q21 *CLCN5*/300008 Xp11.22 *SLC34A3*/609826 9q34	XLH is is the most common disease associated with hyperphosphaturia in infancy. Clinical features include growth retardation with rachitic and osteomalacic bone disease. renal defects in phosphate reabsorption and vitamin D metabolism. Vitamin D treatment do not prevent growth failure. The bone disease is much less severe in females. ADHR: patients frequently present with bone pain, rickets, and tooth abscesses. The penetrance is incomplete and the age at onset is variable (childhood to adult). Resolution of the phosphate-wasting defect has been described in rare cases ARHP: clinical festures very similar to those observed in ADHR, but the inheritance is recessive. X-recessive form presents with rickets, osteomalacia, and early onset lower extremity deformities. It may progress in renal insufficiency during adulthood.	X-liked: oral phosphate supplementation and 1(OH)D$_3$ or 1,25(OH)$_2$D$_3$. This therapy improves but does not cure the bone disease, causes nephrocalcinosis in 60% of the patients and may cause renal failure or hyperparathyroidism. Thiazide diuretics decrease urinary calcium excretion and

		Hypophosphatemic rickets with hypercalciuria: presents with rickets, bone pain, muscle weakness, failure to thrive. *XLH: hypophosphatemia and increased alkaline phosphatase, normal or low serum levels of 1,25-dihydroxyvitamin D_3.* *ADHR: hypophosphatemia, and inappropriately normal 1,25-dihydroxyvitamin D_3 (calcitriol) levels.* *X-recessive: hypercalciuria, hypophosphatemia, nephrocalcinosis, proteinuria.* *Hypophosphatemic rickets with hypercalciuria: hypophosphatemia with hyperphosphaturia, normocalcemia with hypercalciuria, high plasma 1,25$(OH)_2D_3$ concentration, low PTH concentration, and elevated plasma alkaline phosphatase activity.*	prevent progression of nephrocalcinosis. ADHR: phosphate and high doses of vit. D. Hypercalciuric form: supplemental phosphate.
Pseudohypoparathyroidism type IA (PHP IA)/103580 ***Pseudopseudohypoparathyroidism (PPHP)***/612463 ***Pseudohypoparathyroidism type IB (PHP IB)***/603233 ***Pseudohypoparathyroidism type IC (PHP1C)***/612462 ***Pseudohypoparathyroidism type II (PHP II)***/203330	*GNAS*/139320 20q13.2 *STX*/603666 20q13.32, 20q13.2, 20q13.2	PHP is heterogeneous group of disorders characterized by resistance to the *parathyroid hormone*. Its pathogenesis has been linked to dysfunctional *G Proteins* (*Gs alpha subunit* (GNAS)) **PHP IA**: presents with a constellation of developmental and skeletal defects, collectively termed Albright hereditary osteodystrophy (AHO). These features include short stature, rounded face, shortened fourth metacarpals and other bones of the hands, obesity, dental hypoplasia, and soft-tissue calcifications. Mental retardation has been reported in some patients. PHP1A is also associated with resistance to TSH, glucagon and gonadotropins. Alterations may even affect ADH, ACTH, and GH–releasing hormone. GNAS1 mutation is in the maternally-derived allele. **PPHP**: AHO phenotype without endocrine abnormalities. GNAS1 mutation is in the paternally-inherited allele **PHP IB**: renal PTH resistance without AHO features; the Gs activity is normal but an imprinting/methylation defects at the GNAS locus results in lack of expression of the maternal allele in renal tissue. Classically, patients do not have features of AHO. TSH resistance has been reported. **PHP IC**: probably is a variant of PHPIA; the only difference is a normal GNAS activity. **PHP II**: isolated renal PTH resistance in patients without AHO phenotype. The resistance may be transient. The Gs activity is normal. The disease seems to be a heterogeneous clinical disorder secondary to a defect in PTH responsiveness distal to cAMP. *Laboratory findings: hypocalcemia, hyperphosphatemia, inappropriately high PTH.* ***PHP1A**, **PHPIB**, **PHP IC**: decreased urinary cAMP response to PTH infusion and decrease urinary PO4 response to PTH.* ***PPHP**: normal urinary cAMP response to PTH infusion and normal urinary PO4 response to PTH.* ***PPH II**: normal urinary cAMP response to PTH infusion and decreased urinary PO4 response to PTH.*	Symptomatic hypocalcemia needs intravenous calcium administration. Oral calcium and calcitriol should be initiated in every patient with a diagnosis of PHP. The goals of therapy are to maintain serum total and ionized calcium levels within the reference range to avoid hypercalciuria and to maintain PTH levels to normal.
WAGR S./194072	*WT1*/607102 *PAX6*/607108 *BDNF*/113505 11p13	Wilms' tumor, Aniridia, Genitourinary anomalies (ambiguous genitalia or gonadoblastoma of testes or avaries) and mental Retardation. Obesity is present in the subtype WAGRO (mutation of the *BDNF* gene).	–
Williams S./194050	*WBS*/194050 *WBSCR26*/612545 *WBSCR27*/612546 *WBSCR28*/612547 7q11.23	See § 121.7	See § 121.7

* *NHS* National Health Service.

122

Disorders of Thyroid Function

Paolo Cavarzere and Luciano Tatò

122.1 Origins of the Thyroid Gland

The thyroid is a bilobed gland located in the neck region, made up of two types of cells: the follicular cells which produce thyroxine and the parafollicular cells which produce calcitonin. The follicular cells are the predominant cell population in the gland and they derive from the endoderm, whereas parafollicular cells are of neuroectodermal origin [1]. These different types of cells migrate from their respective sites of origin to ultimately merge in the definitive thyroid gland. The follicular cells organize into thyroid follicles whereas the other type of cells scatters into the interfollicular space [2]. Most thyroid function disorders are related to developmental or enzymatic defects involved in the process of thyroid hormone production.

122.1.1 Thyroid Organogenesis

Thyroid embryogenesis begins between the 20th and 22nd days of fetus development with the appearance of the thyroid bud as a thickening in the pharynx floor. It is the first recognizable endocrine structure. On the 26th day of development, the thyroid diverticulum begins its migration towards its definitive pretracheal position which it reaches between the 48th and 50th day of embryonic life. By the 28th day of development, the thyroid is connected to the pharynx by a small pedicle known as the thyroglossal duct [3, 4]. This duct disconnects from the pharynx around the 33th day of development and by the 40th day it disappears completely. In some cases, this structure persists and gives origin to thyroglossal duct cysts during infancy [5]. The gland already has a bilobed shape by the 28–30th day of fetal development. Around the 44th day of development, the two cell populations join together and the parafollicular cells are incorporated into the gland. The formation of thyroid follicles and gene expression involved in hormonogenesis begin when migration ends. Hormone synthesis begins during the l0–12th week of development with the incorporation of iodine into the thyroid hormones [3, 4, 6].

122.1.2 Genetic Regulation

Several factors are involved in the process of thyroid organogenesis, in particular the *Nkx2.1*, *Pax-8*, and *Ttf-2* transcription factors which are mostly thyroid-specific. However, none of them is exclusively expressed in the thyroid gland. In addition to transcription factors, the receptor for thyroid stimulating hormone (TSH) plays an important role in gland morphogenesis. Whereas *Nkx2.1*, *Pax-8* and *Ttf-2* are expressed by thyroid follicular cells from the very start of their differentiation, the TSH receptor (TSHR) takes part in the proliferation and function of the thyroid follicular cells, but it is not required for early organogenesis or for the migration process [2, 7–9]. Mutations in all these genes are related to changes in thyroid morphogenesis; they have been detected in patients with thyroid dysgenesis [10, 11]. In addition, other genes have been found to be expressed during thyroid development in mice models. In particular, *Hhex* seems to be involved at early stages of morphogenesis, whereas *Hoxa3* and *Eya1* seem to be involved in the later stages of thyroid organogenesis [12].

122.2 Function of the Thyroid Gland

122.2.1 Fetal Thyroid Hormone Production

The main function of the thyroid gland is to produce thyroid hormones. In the early period of gestation, the fetus is entirely dependent on maternal transfer of thyroid hormones;

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

consequently, total serum T4 and T3 levels in early gestation are low, depending entirely on the function of the placenta and maternal thyroid status.

During the first weeks of gestation, the human fetal thyroid gland begins to synthesize only minute amounts of T4 and T3. Around the 20th week of gestation, the fetal hypothalamic-pituitary-thyroid axis begins to function and fetal production of TSH and T4 progressively rises to peak in the month before term [13]. Total T3 levels in fetal blood remain low until the 26–30th week of gestation due to the immature activity of the deiodinase type I enzyme which converts T4 into T3. However, during gestation, both deiodinase types III and II are found to be active. The former, of fetal and placental, origin, converts T4 into inactive reverse T3 and T3 into di-iodothyronine. The latter mediates the production of T3 from T4 in selected fetal tissues, especially in the brain, where it is actively transferred by MCT8. The maturation of type I occurs gradually in the fetal liver during the last weeks of gestation, although the levels of T3 rise only moderately around term because placental deiodinase III remains at high levels throughout the gestation period [14–16]. Moreover, plasma thyroid hormone levels are correlated to gestational age; consequently, preterm infants present lower T3, T4 and FT4 values than term infants [17–20].

Finally, it is well known that the limited transplacental passage of thyroid hormones during gestation is sufficient to preserve fetal brain development and its function, in case there is an abnormality in the thyroid ontogenesis [13, 21, 22]. On the contrary, when both maternal and fetal hypothyroidism present, there is significant impairment in neuro-intellectual development despite adequate therapy soon after birth [21]. Maternal hypothyroidism can lead to mild but significant cognitive impairment of the offspring [5, 23, 24].

Table 122.1 Etiologies of congenital hypothyroidism

Permanent
- Thyroid dysgenesis (75-85%)
  - Athyrosis (20%)
  - Ectopy (75%)
  - Hypoplasia, hemiagenesia (~5%)
- Dyshormonogenesis (10–20%)
  - Iodine transport defect (Na/I symporter)
  - Thyroglobulin defect
  - Pendrin defect (Pendred syndrome)
  - TPO defect
  - DUOX2 and DUOXA2 defect
- Resistance to TSH (rare)
  - TSH receptor mutations
  - Pseudohypoparathyroidism Ia (Gα protein alteration)
- Central hypothyroidism (rare)
  - TRH deficiency, resistance to TRH
  - TSH deficiency
  - Mutations in transcription factors implied in pituitary development (*PIT1*, *PROP1*, *LHX3*, *LHX4*, *HESX1*)
  - Hypothalamic-pituitary defect
- Peripheric hypothyroidism (rare)
  - Resistance to thyroid hormone
  - Alteration of thyroid hormone transporter (MCT8 mutation)

Transient
- Iodine deficiency
- Iodine overload
- Maternal anti-thyroid treatment
- Trans-placental passage of blocking TSH antibodies
- DUOX2 heterozygous mutations

122.2.2 Perinatal Thyroid Hormone Modification

At birth, the rapid fall in ambient temperature causes a peak in the newborn TSH level within 30 minutes after delivery, with a consequential increase in FT4 levels. In term infants, FT4 levels fall within 4–6 weeks of life, whereas in preterm newborns, the increase is blunted and related to gestational age. In infants born after 30–32 weeks of gestation, the FT4 peaks slowly and is comparable to that of term babies; on the contrary, preterm infants born before 30 weeks or with low (LBW) or very low birth weight (VLBW) present a decline in FT4 with a peak at 1–2 weeks of life, without an increase in TSH [19, 25].

122.3 Congenital Hypothyroidism

122.3.1 Epidemiology

Congenital hypothyroidism (CH) has an incidence of 1:3500 live births in iodine-sufficient regions [26]. CH is more frequent in females than in males (ratio 2:1); infants with Down syndrome have a higher risk of developing CH [27].

122.3.2 Etiology

Whereas the hypothalamic-pituitary-thyroid axis maintains a stable concentration of FT4 in normal subjects, in children with hypothyroidism there is an under-function of the gland with an increase in TSH concentrations in an attempt to compensate for the hormone deficiency (Table 122.1). Exceptions to this mechanism are central hypothyroidism and other rarer conditions.

122.3.2.1 Thyroid Dysgenesis

Thyroid dysgenesis is the most frequent cause of CH [28]. It includes athyrosis, due to the complete absence of thyroid tissue; ectopic gland, due to an abnormal migration of the embryonic thyroid; and thyroid hypoplasia, due to defective

growth of the gland following complete migration. The most frequent type of thyroid dysgenesis is ectopy; the majority of these patients presents a thyroid in the dorsum of the tongue or, less frequently, located in a sublingual position [29].

Thyroid dysgenesis can be correlated to a mutation in the *Nkx2.1*, *Ttf-2* and *Pax-8* genes, as mentioned above [10, 11]. In particular, *Nkx2.1* mutations are associated with predominant neurological phenotype, pulmonary lesions and thyroid dysfunction [30, 31]; homozygous mutations of *Ttf-2* have been related to athyrosis, cleft palate and choanal atresia, whereas heterozygous conditions lead to euthyroidism [32]; finally, mutations in the *Pax-8* gene have been detected in isolated thyroid phenotypes in some cases associated with renal anomalies [33–35].

122.3.2.2 Thyroid Dyshormonogenesis

When thyroid gland is in situ, the disorder may be related to inborn errors of metabolism during one of the several steps required for normal thyroid hormone synthesis (thyroid dyshormonogenesis). Different mutations were reported in the genes encoding the thyroglobulin protein, the sodium iodide symporter and the pendrin channel, as well as in the thyroid peroxidase (TPO), and more recently in the dual oxidases 2 (DUOX2) enzyme and its maturation factor DUOXA2 [36–46].

Whereas thyroid dyshormonogenesis invariably displays a recessive mode of inheritance, thyroid dysgenesis is usually sporadic (only 2% of familial cases) [47].

122.3.2.3 Resistance to TSH

In this rare condition, the response to bioactive TSH may be impaired at the thyroid follicular cell level and the cause could be found in mutations that inactivate the TSHR [9]. Total insensitivity to TSH leads to a small orthotopic gland with a phenotype ranging from CH to euthyroid hyperthyrotropinaemia [48]. Rarely, patients with pseudohypoparathyroidism Ia also present resistance to glycoprotein TSH, LH and FSH hormones [49]. Unresponsiveness to TSH has also been described as an autosomal dominant condition associated with unknown molecular defects [50].

122.3.2.4 Central Congenital Hypothyroidism

Central CH occurs in 1 out of every 50,000 newborns. It is often related to abnormalities during development of the hypothalamus or the pituitary glands and associated with multiple pituitary hormonal deficiencies. Rarer causes of central CH may be ascribed to TRH deficiency, resistance to TRH, isolated TSH deficiency related to mutations in the TSH-β chain and mutations in transcription factors involved in pituitary development and hormone expression [9, 51–53].

Central CH was also described in babies born from mothers with gestational hyperthyroidism. One cause could be insufficient treatment of the mother during pregnancy and, consequently, the hyperthyroid environment that impairs maturation of the fetal hypothalamic-pituitary-thyroid axis [54].

122.3.2.5 Peripheric Hypothyroidism

This rare form of CH can be due to tissue resistance to the action of thyroid hormones, caused by inactivating mutations of the β-receptor of triiodothyronine or to abnormalities in thyroid hormone transport across the cell membrane, as in MCT8 mutations [55]. In the first condition, free thyroid hormones increase and TSH is inappropriately normal or higher; in the second case, FT3 is higher, whereas FT4 is low and TSH is normal or increased. Clinically, children affected by the first condition show a variable phenotype ranging from isolated biochemical abnormalities to variable features of hypothyroidism; those affected by the second condition present severe neurological symptoms with major hypotonia and delay of acquisitions [56].

122.3.2.6 Transient Congenital Hypothyroidism

CH is sometimes transient and often has an environmental or iatrogenic origin. Transient CH may be related to a deficiency of iodide or to exposure to an excess of iodine, usually due to the application of iodinated disinfectants on the skin [57]. Other causes are treatment with anti-thyroid drugs and, more seldom, the trans-placental crossing of antibodies blocking the action of TSH [58]. Recently, heterozygous mutations in the DUOX2 gene have been found in patients with transient CH [44].

122.3.3 Clinical Signs

The trans-placental passage of maternal thyroid hormone protects newborns with CH for about 2 weeks [59]; consequently, at birth, only 1–4% of newborns with CH are detected by clinical examination [60]. Infants with CH are usually overdue, have a high birth weight for their gestational age [61]. Other signs of CH are represented in Table 122.2.

Children with central CH often have clinical manifestations of other associated hormonal deficiencies such as hypoglycemia, micropenis and cryptorchidism [58]. Malformations of the median line have also been associated with central CH. Cardiac malformations represent the most frequent birth defect associated with thyroid dysgenesis, although the influence of heart development on thyroid organogenesis has not been demonstrated yet [62].

Table 122.2 Symptoms of congenital hypothyroidism

- Prolonged jaundice
- Skin mottling
- Large anterior and/or posterior fontanel
- Abdominal distension
- Umbilical hernia
- Hypotonia
- Constipation
- Poor feeding
- Failure to thrive
- Hoarse cry
- Hearing impairment
- Neurodevelopmental delay
- Irreversible mental retardation (in absence of precocious diagnosis)
- Goiter (rare at birth)

122.3.4 Neonatal Screening

The diagnosis of CH is made through mass neonatal screening within the 5th day of life. The purpose is to prevent mental retardation due to CH. As a screening method, it can be used as a primary T4 strategy or, more frequently, as a primary TSH strategy or a combined primary approach, which is the ideal screening approach [63].

Screening should be done between 48 hours and 4 days of life, in any case before discharge from the nursery. It must be performed before a transfusion or the diagnosis could possibly be missed. In critically ill or preterm babies, blood for screening should be obtained within 7 days of life. Infants in the Neonate Intensive Care Unit usually have more urgent medical problems, but in any case the sample must be collected before discharge or a transfer to another hospital. Nowadays, a negligible number of infants with CH are missed by newborn screening programs [64]. This deficiency in the screening program is often related to errors in specimen handling, testing, data analysis or result reporting. In other cases, the TSH increase can occur later, usually in preterm infants, because of the immature function of the hypothalamic-pituitary axis [64–66].

122.3.5 Procedures to Confirm Diagnosis

All newborns with abnormal results at screening for CH must undergo FT4, FT3 and TSH serum measurements to confirm the diagnosis; newborns with hypothyroidism typically present low FT4 and high TSH concentrations [64–66]. In addition, it is imperative to make TSH and FT4 measurements as quickly as possible in all cases of clinical suspicion of hypothyroidism, regardless of screening results.

Etiological diagnosis of CH is based on scintigraphy, ultrasound analysis and measurements of thyroglobulin serum. High thyroglobulin levels may suggest dyshormonogenesis, absent levels an athyreosis [60, 64, 67]. In addition, serum and urinary iodide, as well as tests for thyroid autoimmunity (anti-peroxidase, anti-TSH receptor, and anti-thyroglobulin antibodies), are helpful in making a complete etiologic diagnosis [64, 68, 69].

122.3.5.1 Thyroid Scintigraphy

Iodine-123 scintigraphy of the thyroid gland is helpful in obtaining information about the function of the thyroid gland. More frequently scintigraphy is performed with Pertechnetate, which is easily available [70]. This allows diagnosis of an ectopic gland and enables the identification of the exact area of the contrast.

The absence of iodine or pertechnetate allows the diagnosis of athyreosis or, in the presence of high doses of thyroglobulin, the diagnosis of mutations that inactivate the TSHR, NIS mutation or maternal antibodies against TSH [71]. When scintigraphy shows a normally located thyroid gland a positive perchlorate discharge test will enable the diagnosis of iodine organification defects [72].

122.3.5.2 Thyroid Ultrasonography

Thyroid ultrasonography is helpful in defining the structure of a normally positioned gland. It is less useful in making a diagnosis because it does not allow the diagnosis of an ectopic gland [73]. On the other hand, fetal ultrasound can make a prenatal diagnosis of dysgenesis or fetal goiter [74]. At present, the best approach for a postnatal diagnosis of CH is to do both an ultrasound, to determine thyroid-gland size and morphology, and scintigraphy, to detect functional thyroid tissue [75].

122.3.5.3 Further Diagnostic Tools

At diagnosis of CH, the absence of one knee's epiphyses at radiography is a marker of fetal origin of the disease and therefore an indication that the infant is at a higher risk of developmental retardation. Serum and urinary iodide measurements aid in determining iodide deficiency or excess. Tests for thyroid autoimmunity allow the physician to associate CH with trans-placental passage of maternal antibodies [76–79].

122.3.6 Therapy

Treatment with L-thyroxine must be initiated as soon as possible [80]. The goal of the therapy is to normalize T4 levels within 2 weeks and TSH values within 1 month. This way, it

is possible to assure the normal growth and development of babies with neuro-cognitive outcome similar to the child's genetic potential. An initial dosage of 10–15 μg/kg/die is recommended [64]. In general, patients with athyreosis require higher doses compared to patients with ectopy, and these require higher doses compared to children with CH due to dysormonogenesis [81].

The drug must be taken in the morning at least 30 minutes before breakfast. L-thyroxine tablets should be crushed and mixed with a few millilitres of liquid to prepare the daily dose. Several substances such as soy protein, iron and calcium are reported to interfere with thyroxine. There have been no proven advantages so far to the association of L-thyroxine with triiodothyronine treatment [82].

If permanent hypothyroidism has not been confirmed, it is advisable to stop the therapy after 3 years and to wait 4 weeks and reassess thyroid function [83]. If it is normal, transient hypothyroidism is presumed and treatment can be permanently interrupted. However, the interruption of treatment must be limited to infants with a normally developed or enlarged thyroid gland that shows, during replacement therapy, TSH levels in the normal range.

122.3.7 Follow-up

Newborns with CH must be assessed after 2 and 4 weeks from the start of therapy, then every 1–2 months for the first 6 months of life and later on every 3–4 months until the first year of life. After that age, a follow-up check-up must be done every 6 months until 3 years of life, then every year [60, 64]. During follow-up, the dosage of the drug must be changed in relation to TSH and FT4 levels, especially during the first 3 years of life when growth is most rapid. Serum FT4 and TSH should be monitored every 4 weeks after any change in L-thyroxine dosage. FT3 measurements are not helpful in monitoring the treatment because this test may be normal despite low FT4 and high TSH levels [59].

In the first months of life it is essential to be aware of possible prolonged hyperthyroidism that can be associated with premature craniosynostosis. Four or more episodes of insufficiently suppressed TSH after the age of 6 months is the most important variable associated with poor school performance [84]. At follow-up visits, regular height and weight must be observed. Delayed bone age usually becomes compatible with the actual age within the third year of life [60].

Neurodevelopmental delay is not common in patients with CH in therapy from the first month of life [85]. Nevertheless, it is possible to notice slight difficulties in motor skills and impairment of visuospatial processes and selective memory, presumable signs of poorly compensated fetal hypothyroidism [86, 87]. However, children with properly treated CH do not show differences in school performance compared with healthy children [88, 89].

122.4 Thyroid Dysfunctions in Preterm Infants

122.4.1 Thyroid Function in Preterm Infants

Alterations of thyroid function in preterm infants are often transient and, in most cases, do not require any treatment. The function of the hypothalamic-pituitary-thyroid axis in preterm newborns is attenuated at birth for an unknown postnatal duration. In preterm babies there is reduced hypothalamic TRH production, an immature response of the thyroid gland to TSH, an inefficient capacity of the thyroid follicular cells to organify iodine and a low capacity to convert T4 into active T3. The responses of TSH and T4 to TRH are normal, so the site of immaturity seems to be the hypothalamus [90]. Moreover, neonatal health conditions related to preterm delivery, such as respiratory distress, can influence serum thyroid hormone levels [91].

122.4.2 Hypothyroxinemia of Prematurity

Approximately 12% of preterm infants present low T4 and FT4 with normal TSH values. This condition is known as transient hypothyroxinemia of prematurity [92]. The cause is not clear; we can hypothesize an immaturity of the hypothalamic axis, the withdrawal of maternal-placental T4 transfer, developmental constraints on the synthesis, immature peripheral metabolism of iodothyronines, iodide deficiency and non-thyroidal illness. Associations have been shown between transient hypothyroxinemia and prolonged oxygen supplementation, mechanical ventilation, intraventricular hemorragia and cerebral white matter damage [93–95]. Bacteremia, persistent ductus arteriosus, necrotizing enterocolitis and the use of drugs such as aminophylline, caffeine, glucocorticoids and dopamine have also been found to be significantly associated with transient hypothyroxinemia [96]. This condition is present in the majority of infants born under 30 weeks of gestation and can be associated with later neurodevelopmental deficits [97]. However, it remains unclear whether morbidity and developmental disabilities are caused by, or simply associated with, hypothyroxinemia of prematurity [25, 98]. Treatment with L-thyroxine has not been shown to be beneficial; on the contrary, it may be detrimental. Presently, in the absence of high TSH, the evidence does not support a cognitive benefit of L-thyroxine treatment for this condition in premature infants [25, 99, 100].

122.4.3 Hypothyroidism with Delayed TSH Elevation

Some LBW or VLBW newborns or critical ill neonates frequently present CH characterized by low FT4 and delayed

TSH elevation. The incidence of this condition is 1:250 for VLBW babies and 1:1589 for LBW newborns. Serum TSH in these babies increases during the first few weeks of life until concentration levels are typical of primary hypothyroidism. It is not known if this type of CH is transient or permanent, or whether it depends on an abnormality of pituitary-thyroid feedback regulation. If TSH is still high at 6 weeks of age, the infant must begin therapy [64].

122.4.4 Transient Congenital Hypothyroidism

Approximately 5% of preterm infants admitted to neonatal intensive care present a transient form of CH with low FT4 and increased TSH [101]. Causes vary from maternal exposure to antithyroid drugs to maternal TRB antibody status. There is evidence suggesting a relationship between this clinical finding and the exposure to iodine-containing antiseptics used in neonatal units. The therapy should begin immediately just as it is for permanent CH; an attempt should be made later in life to suspend treatment. On the contrary, the hormone levels in babies born from mothers in treatment with antithyroid drugs usually return to the normal range within 1–3 weeks without therapy [64].

122.5 Asymptomatic Neonatal Hyperthyrotropinemia

Asymptomatic hyperthyrotropinemia is defined as a high serum TSH level associated with normal T4, FT4 and T3 values [64]. This subclinical condition, transient or permanent, is relatively frequent with a prevalence of 1:8000 newborns in Europe, and it is more frequent in infants with Down syndrome [102].

122.5.1 Diagnosis

Affected newborns are detected by neonatal screening. About 60–70% of infants with increased TSH levels at neonatal screening present normal or nearly normal serum TSH values with normal FT4 levels and require follow-up during childhood [102, 103].

122.5.2 Etiologies

The cause is not known, though it is possible that the same factors involved in the origins of CH, when they are less severe, are responsible for subclinical hypothyroidism. Some studies have identified genetic abnormalities, thyroid gland malformations and autoantibodies in children with hyperthyrotropinemia [103]. These defects often present different degrees of severity and therefore could increase TSH concentrations moderately and intermittently [103]. This clinical finding appears more evident at birth because the thyroid gland is less mature and there is a greater thyroid hormone requirement during neonatal life. Other causes of asymptomatic hyperthyrotropinemia are defects in the biological activity of TSH or of its receptor, subtle developmental defects such as hemithyroid, and a disturbance in the TSH feedback control system [66].

122.5.3 Evolution

Repeated thyroid function evaluation is essential because TSH values in the same individual can spontaneously fluctuate around the upper normal range [104]. With age this condition can become clinically evident as the disease progresses, as in overt hypothyroidism [105]. In other cases, high TSH values in very young children may subsequently normalize; perhaps the cause is due to reduced thyroid hormone requirements in more advanced childhood [102, 103]. In any case hyperthyrotropinemia requires periodic follow-up visits to assess thyroid gland function.

122.5.4 Treatment

Whether subclinical hypothyroidism requires treatment or not is still a matter of debate also because only few studies have investigated the effect of L-thyroxine therapy in children with subclinical hypothyroidism. Nevertheless, there is a general consensus to treat all subjects with TSH levels over 10 mU/L and normal FT4 [103]. Over-treating must be avoided and the therapy discontinued after 3 years of age in order to reassess thyroid function [64, 106].

122.6 Neonatal Hyperthyroidism

Neonatal hyperthyroidism is a relatively rare condition (incidence varying between 1:4,000 and 1:40,000) that could be due to maternal thyroid autoimmune disorders, to continually activated TSH receptor or to McCune-Albright syndrome.

122.6.1 Graves' Disease

The most frequent cause of neonatal hyperthyroidism is the mother suffering from Graves' disease, which occurs in

0.1–0.4% of pregnant women. However, only 1% of neonates born to these affected women presents hyperthyroidism and in all cases this form of disease is transient [107].

122.6.1.1 Etiology

The transplacental passage of maternal immunoglobulin stimulates the fetal thyroid by activating the TSH receptor. Consequently, thyroid hormone secretion in the uterus is already increased by thyrotoxicosis that continues after birth until the maternal antibodies have disappeared from the infant's circulation [108]. This fetal condition begins in the second trimester of gestation when fetal TSH receptor begins to respond to TSH stimulation; it is related to the severity of the mother's disease and goes away within 4 months of postnatal life [108].

122.6.1.2 Clinical Signs

Thyrotoxic fetus may present growth restriction, fetal tachycardia, increased fetal motility, goiter and hydrops [90, 109, 110]. These are frequently premature births [111]. In other cases the symptoms are only noticed at birth or several days afterward when the effect of maternal drugs have disappeared, and the maternal immunoglobulins, which have a longer half-life, have increased thyroid hormone production allowing the clinical signs of the disease to become evident [112]. The neonatal symptoms vary from tachycardia and hyperexcitability to increased appetite with poor weight gain, vomiting or diarrhea, fever with sweating or erythema, tachypnea in some cases and goiter in 50% of newborns [90, 107, 109] (Table 122.3). Often the goiter is the first sign of thyroid dysfunction and already appears during fetal life [113]. Mortality has been reported to be between 12–20% [114]. Heart failure is one of the major risks; consequently, early diagnosis and treatment are necessary for a good prognosis [109]. In thyrotoxic newborns, jaundice, cholestasis and thrombocytopenia are also indicated as signs of the disease. Hepatomegaly and splenomegaly can be present and liver function abnormalities can occur even in the absence of heart failure [90, 109]. Stare and eyelid retraction are present, whereas exophthalmia is related to the autoimmune process [115]. The fontanels are small and some infants show craniosynostosis; bone maturation is often advanced as a result of fetal hyperthyroidism [90, 109].

Table 122.3 Signs and symptoms of congenital hyperthyroidism

- *Fetal signs*
  - Growth restriction
  - Fetal tachycardia
  - Goiter
  - Idrops
  - Premature births (*frequent*)
  - Increased fetal motility
- *Neonatal signs*
  - Tachycardia
  - Hyperexcitability
  - Increased appetite with poor weight gain
  - Vomiting
  - Diarrhea
  - Fever with sweating or erythema
  - Tachypnea
  - Goiter (50% of newborns)
- *Thyrotoxic signs*
  - Heart failure
  - Jaundice
  - Cholestasis
  - Thrombocytopenia
  - Hepatomegaly
  - Splenomegaly
  - Liver function abnormalities (also in absence of heart failure)
  - Stare and eyelid retraction
  - Exophthalmia
  - Small fontanels
  - Craniosynostosis
  - Advanced bone maturation

122.6.1.3 Diagnosis of Fetal Hyperthyroidism

Diagnosis of prenatal hyperthyroidism is based on high maternal TSH receptor binding antibody titers, accelerated fetal bone maturation and a fetal heart rate over 160 bpm, in the last part of gestation [108]. If a fetal goiter is present, it is necessary to determine whether the cause is hyperthyroidism related to maternal Graves' disease or hypothyroidism related to maternal anti-thyroid drugs. To make a correct diagnosis, it is helpful to use a Doppler of the fetal thyroid which indicates hypothyroidism when it shows a flash confined to the periphery of the gland [116]. Finally, a fetal blood sample is the gold standard to differentiate fetal hypothyroidism from fetal hyperthyroidism, but it is not easy to obtain and can be risky for abortion and fetal infection [109, 117]. Assessment of fetal thyroid hormones during pregnancy must be done only in cases when the status of the fetus is in doubt, the mother has positive TSH receptor binding antibody titers and takes anti-thyroid drugs and when intra-amniotic L-thyroxine therapy may be necessary to resolve the fetal clinical condition [110]. Fetal FT4 levels are related to maternal FT4 values: a fetal euthyroidism can be achieved by maintaining maternal FT4 in the upper normal-to-mild thyrotoxic range during treatment with anti thyroid drugs [109, 115].

122.6.1.4 Diagnosis of Neonatal Hyperthyroidism

The diagnosis of neonatal hyperthyroidism is confirmed by high FT4 and FT3 levels with low values of TSH. Since a delayed appearance of hyperthyroidism is possible, these tests should be repeated a few days after the first serum test [90, 109]. The determination of antibodies is a useful marker of the etiology of the disease [115].

122.6.1.5 Treatment

It is essential to treat newborns for hyperthyroidism soon after birth in order to avoid short-term and long-term morbidity [107]. Propylthiouracil, which promptly restores a normal heart rate, is the drug of choice for this condition. It should be administered orally at a dosage of 5–10 mg/kg/day divided into three doses [90, 109]. Its advantages are to block the secretion of thyroid hormones, blocking iodide organification and the coupling of iodothyronine residues, and to decrease the peripheric conversion of T4 to T3 [118].

In place of propylthiouracil, methimazole may also be used to block thyroid hormone secretion.

It is less frequently used to treat neonatal hyperthyroidism; when it is used, the dosage is 0.25–1 mg/kg/day divided into two doses [119]. Propranolol, which inhibits deiodination of T4 to T3 and controls symptoms caused by adrenergic stimulation, may be given at a dosage of 1–2 mg/kg/day divided into three doses when tachycardia is present [119]. In the most severe cases of the disease, a saturated solution of potassium iodide (1 drop/day) or Lugol's solution (1–3 drops/day) can be added in order to decrease thyroglobulin proteolysis and thyroid hormone production. Sodium iopanoate (iopanic acid, 500 mg orally once every third day) and glucocorticoids (prednisone 2 mg/kg/day) are rarely used to decrease thyroid hormone production and to inhibit T4 conversion to T3 [120].

Other helpful measures such as high liquid and calorie intake and sedatives are also useful in the treatment of these newborns [90, 109]. The therapy should be continued until maternal antibodies are no longer present. During treatment, the patients must be seen weekly until they are stable and then every 1–2 weeks, reducing the dosage as soon as possible. In some cases, it might be helpful to treat the newborns with fixed doses of anti-thyroid drugs and to add L-thyroxine when plasma FT4 levels reach the hypothyroid range. This combined treatment is usually required for 1 month [107].

Treating the mother with propylthiouracil or methimazole, at a dosage under 150 mg/day and 30 mg/day respectively, does not mean breast-feeding should be discontinued since the quantity of drugs that the newborn would ingest is minimal; however, it is necessary to do periodic assessments of neonatal thyroid function [121]. Fetal hyperthyroidism must be treated with maternal anti-thyroid drugs and in this context propylthiouracil is preferred because of what has been reported on carbimazole and methimazole embryopathy [122].

122.7 Mutations of TSH Receptors

Activating mutations of the TSH receptor cause constitutive activation of the receptor, which is not related to an autoimmune mechanism and is dominantly transmitted [123]. The phenotype appears with neonatal hyperthyroidism. This permanent condition is often associated with a severe course with moderate or diffuse goiter due to the mitogenic effects of cyclic AMP on thyroid follicular cells, possible developmental delay, absence of extrathyroidal signs of Graves' disease and recurrence after treatment. Sporadic congenital non-autoimmune hyperthyroidism may be also caused by de novo mutation of the TSH receptor gene. Definitive treatment with a thyroidectomy and radioiodine therapy is possible at older ages [114].

122.7.1 McCune-Albright Syndrome

McCune-Albright syndrome results from an activating mutation in the gene encoding the α-subunit of the stimulatory G proteins; in some cases its symptoms are similar to neonatal hyperthyroidism. Clinically besides symptoms of hyperthyroidism, cafè-au-lait spots are present with irregular borders, polyostotic fibrous dysplasia and other endocrinological alterations. The medical treatment is the same as Graves' disease but it must be prolonged during postnatal life. Once euthyroidism has been restored, an ablative approach is possible [124].

122.7.2 Hashimoto's Thyroiditis

Maternal Hashimoto's thyroiditis may cause hyperthyroidism in the newborn but usually there are no consequences in the fetus, even though antibodies against TPO or thyroglobulin can cross the placenta and be found in the fetus. However, cases of fetal and neonatal hyperthyroidism have been found in the offspring of affected mothers [125].

122.7.3 Resistance to Thyroid Hormones

Resistance to thyroid hormones is a rare condition characterized by decreased responsiveness to thyroid hormones. High FT3 and FT4 are present with inappropriately normal or elevated TSH concentrations [126]. Clinically, the affected patients show a variable phenotype ranging from isolated biochemical abnormalities to variable features of hypo- or hyperthyroidism in the rare case of pituitary resistance [127]. Asymptomatic patients do not require any treatment whereas therapy must be initiated to resolve the symptoms of hypo- or hyperthyroidism.

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123

Disorders of Sexual Development

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123.1 Sex Determination and Differentiation

123.1.1 Sex Development

It is rare to be unable at birth to differentiate a male from a female newborn because of the presence of ambiguous genitalia, but abnormalities of the external genitalia that need further investigation may occur in about 1 in 4000 births [1]. Antenatal ultrasound scanning has improved our ability to predict these conditions.

Sex development is a genetically and hormonally controlled process, which in humans starts immediately at fertilization by the establishment of the chromosomal sex (XX or XY). This is the first step of a process of sex determination which continues with the formation of the gonad (testis or ovary). Following this, sex determination depends on the sex-chromosome complement of the embryo and is established by multiple genetic and molecular events that direct the development of germ cells, their migration to the urogenital ridge, and the formation of either a testis in the presence of the Y chromosome (46,XY), or an ovary in the absence of the Y chromosome and the presence of a second X chromosome (46,XX).

The second step in sex development concerns sex differentiation, which refers to the formation of the phenotypic sex (appearance of the external genitalia and internal annexes) and the subsequent acquisition at puberty of secondary sex characteristics and reproductive potential. This process depends on the sex-specific response of tissues to hormones produced by the gonads after they have differentiated in a male or female pattern.

Sex outcome at birth is thus the result of a coordinated and sequential series of developmental events controlled by a system of temporally expressed genes, transcription factors and hormones. Any condition affecting this complex pathway may result in a disorder of sex differentiation and a child with an indeterminate genetic, gonadal or phenotypic sex.

123.1.2 Gonads

In humans, bipotential gonads begin their development at around 4–5 weeks' gestation, separating from the adrenal primordium. They arise as paired condensations of the coelomic epithelium at the medioventral region of the mesonephros on either side of the dorsal aorta. The gonad contains both somatic and germ cells. The primordial germ cells, which migrated into the gonads from the yolk sac, generate the gametes (spermatocytes or ova). Until about 40 days' gestation the primitive gonads remain bipotential. The presence, or absence, of testicular determining genes depends on chromosomal sex and determines whether the gonads differentiate into testes or ovaries.

Four major cell types are present in the indifferent gonads: supporting, steroidogenetic, germ lineage and connective cells that will differentiate:

- In the testes as: Sertoli cells, Leydig cells, spermatogonia and peritubular myoid cells, respectively. The Sertoli cell is the first cell type that differentiates to produce the anti-müllerian hormone (AMH) and its role is essential for the nutrition of germ cells; the Leydig cells are steroidogenic cells that produce testosterone. Both hormones serve as an essential step for the normal progression versus male fetal development.
- In the ovary as: granulose/follicular cells, steroid producing cells (theca cells produce androgens, that are converted to estrogens in the granulose cells, that also produce progesterone), oocytes and stromal cells, respectively. Ovarian differentiation starts 1 week later than testis differentiation and its steroidogenic function seems to be of little importance prior to puberty.

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123.1.3 Internal Ducts

At 6 week's gestation both the wolffian ducts (from which arises the seminal vesicles, the vas deferens and the epididymis) and the müllerian ducts (from which arise the fallopian tubes, the upper vagina and the uterus) are present. In normal male differentiation, the regression of the müllerian structures and the inhibition of FSH is dependent on AMH and inhibin B (InB) production through Sertoli cell activity. Neonatal pituitary luteinizing hormone (LH) and placental human chorionic gonadotropin (hCG) stimulate Leydig cells to produce testosterone, indispensable to stabilize the wolffian derivates, while dihydrotestosterone (DHT), deriving from peripheral conversion of testosterone by 5-α-reductase, acts to complete virilization of the external genitalia and the scrotal migration of testes.

In normal female differentiation, the lack of testosterone and AMH leads to regression of the wolffian structures and development of müllerian derivatives. However, recent studies of gene expression have shown that there is a series of specific genes actively implicated in ovarian development and integrity, rendering the latter process more active than previously hypothesized [2].

123.1.4 External Genitalia

The external genitalia are initially identical, regardless of the genetic or gonadal sex and consist of a genital tubercle, urogenital folds and the genital swellings. Male differentiation occurs when sufficient amounts of testosterone and DHT is produced from the 7–8th weeks of gestation, and when the fetus has developed sensitivity to androgens. In this case the genital tubercle develops as a penis, the urethral folds fuse to create a tubular penile urethra with the meatus located at the tip of the penis, and the genital swellings join to form a scrotum.

If androgenic effects are lacking, the genital tubercle forms a clitoris, and the urethral folds and the genital swellings develop into the labia minora and majora, respectively. Finally, in the absence of AMH production, a normal vagina is formed [3].

123.1.5 Psychosexual Development

Psychosexual development refers to a number of distinct components that comprise whether a person views themselves as a man or woman (gender identity), whether that person is viewed by the other members of the society as masculine or feminine (gender role), and their choice of sexual partner and erotic interest (heterosexual, bisexual, homosexual) including behavior, fantasies and attractions (sexual orientation).

123.1.6 Understanding Normal Sex Development by Genetic Advancements

Sex development requires the interaction of several factors together with the production of hormones and their consequent signalling action. It is therefore not surprising that an increasing number of genes have been discovered that contribute both early and late to the process of sex determination and differentiation, and there will undoubtedly be more. Knowledge of the molecular basis of these pathways will influence the diagnosis and management of these conditions. Transcription factors and signaling molecules play an important role in the formation and differentiation of the gonad, processes which are genetically determined. In contrast, genes that cause postgonadal 46,XY or 46,XX disorders of sexual development (DSD) often encode enzymes or factors necessary for sex steroid biosynthesis or action.

123.2 Nomenclature and Classification

Terms like intersex, hermaphroditism or pseudohermaphroditism have been considered unacceptable by affected individuals and support groups. Thus, a new nomenclature has been proposed [4, 5]. The inclusion of congenital in the definition of DSD excludes disorders such as precocious or delayed puberty. Table 123.1 outlines the nomenclature and the classification system based on causes, workable in clinical practice. According to karyotype, DSD are divided in disorders with normal or abnormal sex chromosomes. In the two groups with normal karyotype, the causes of DSD are subdivided into disorders of gonadal development or post-gonadal disorders (hormonal or receptor defects, syndromic forms, etc). In 2008 a European consortium on DSD was created (EuroDSD, www.eurodsd.eu), based on a research project supported by the EU Commission within the 7th Framework Programme. It is a collaborative project involving clinical and genetic experts. At the heart of EuroDSD is a European Registry, that is a targeted virtual environment supporting the sharing of data with respects to security and ethics. The project links patients data collection and analysis tools with research on development of novel diagnostic strategies to identify new causes of DSD, in conjunction with a strong programme on functional molecular biology of the androgen receptor, thereby allowing for an in-depth analysis of a key factor in the pathogenesis of DSD. EuroDSD also provides for an e-learning environment [6].

123.3 DSD with Karyotype Abnormalities

These disorders include diseases in which the number of sex chromosome is impaired (Table 123.2). The first two of these

Table 123.1 DSD classification (Chicago Consensus Conference, 2006)

Sex Chromosome DSD	46,XY DSD		46,XX DSD	
A: 47,XXY (Klineflter syndrome and variants)	A: Disorders of gonadal (testicular) development	1. Complete or partial gonadal dysgenesis (*SRY*, *SOX9*, *SF1*, *WT1*, *DHH*, etc.) (Swyer syndrome)	A: Disorders of gonadal (ovarian) development	1. Testicular dysgenesis (*SRY*+, dup *SOX9*)
		2. testis regression		2. Gonadal dysgenesis
		3. ovotesticular DSD		3. Ovotesticular DSD
B: 45,X0 (Turner syndrome and variants)	B: Disorders of androgen synthesis or action	1. Androgen biosynthesis/ metabolism defect (17-OH-steroid-dehydrogenase deficiency, 5α-reductase deficiency, StAR mutations, etc.)	B: Androgen excess	1. Fetal (21α-hydroxylase deficiency, 11α-hydroxylase deficiency, glucocorticoid receptor mutations)
		2. Androgen action defect (CAIS, PAIS, drugs and environmental modulators)		2. Feto-placentar (aromatase deficiency)
		3. LH-receptor defect (Leydig cell hypoplasia or aplasia)		3. Maternal (luteoma, androgenic drugs, etc.)
		4. AMH or AMH-receptor defect (persistent müllerian duct syndrome)		
C: 45,X0/46,XY (mixed gonadal dysgenesis, ovotesticular DSD)	C: Other	Syndromic associations, hypospadias, cloacal extrophy, cryptorchidism, vanishing testis syndrome, etc.	C: Other	Syndromic associations, MURCS (müllerian aplasia, renal aplasia and cervico-thoracic somite dysplasia), cloacal extrophy, vaginal atresia, etc.

Table 123.2 Sex chromosome DSD

Condition	Karyotype	Gonad	Müllerian structures	External genitalia	Possible neonatal features
Klinefelter's S.	47,XXY and variants	Hyalinized testes	–	Male	Micropenis, hypospadias, cryptorchidism
Turner's S.	46,X and variants	Streak gonad or immature ovary	Uterus	Female	Lymphedema, web neck, cardiac defect and coartation of the aorta, renal and urinary abnormalities
Mixed gonadal dysgenesis	45,X/46,XY and variants	Testis or dysgenetic gonad	+/–	Female, ambiguous, male	Possible presence of some features of Turner's syndrome; increased risk of gonad tumors
Ovotesticular DSD	46,XX/46,XY chimerism Africa 46,XX; rare 46,XY	Dysgenetic testis	+/–	Ambiguous	Possible increased risk of gonad tumors

Tables 123.2, 3, 5, 6, 7 have been modified from Achermann JC, Hughes IA (2007) Disorders of sex development. In: Kronenberg H, Melmed S, Polonsky K, Larsen PR (eds) Williams Textbook of Endocrinology, 11th edn. Saunders Elsevier, Philadelphia.

conditions will generally escape neonatal diagnosis and will only be diagnosed in late pubertal or adult life due to related features [7], e.g., impairment of puberty, or infertility. However, a recent paper by Lee et al [8] reported seven Klinefelter patients (KS) (47,XXY and variants; incidence between 1:500 and 1:1000 live births [9]) with abnormalities of genitalia ranging from mild anomalies (chordee) to moderate undervirilization (bifid scrotum and perineal hypospadias). In the same paper, a review of other cases revealed a range of mild-to-severe abnormalities that underline the importance of recognizing this condition as one of the causes of abnormal genitalia at birth. Early diagnosis and treatment might improve the quality of life of men with KS since they are born with spermatogonia and lose large numbers of germ cells during puberty. The classic form of Turners' syndrome (TS) (about 1:2,500 live birth [10]) is characterized by a 45,X0 karyotype and occurs in about 50% of cases; mosaic forms (45,X/46,XX) and forms with structural anomalies of the X chromosome are

each found in 25% of cases. None of these show genital ambiguity. However, a prenatal diagnosis of TS may be made incidentally (advanced maternal age, detection of increased occipital translucency on fetal ultrasound scan) following amniocentesis (AC) or chorionic villous sampling (CVS). During the neonatal period the diagnosis may be suspected in females with lymphoedema, occipital folds, low hair line, or left sided heart defects. Girls with classic TS have ovarian dysgenesis, an observation which underlines the need for two copies of the X chromosome for regular ovarian development. Gonadectomy is indicated for TS patients when there is a Y fragment containing the *TSPY* locus because of the increased risk of developing gonadoblastoma.

The true prevalence of classic mixed gonadal dysgenesis (MGD) (45,X/46,XY mosaicism) is unknown. The clinical phenotype of this condition is highly variable and cases described in the literature are mainly the most severe forms. The genital phenotypes vary from normal female external genitalia or mild clitoromegaly to all stages of ambiguous genitalia including hypospadias or a normal penis [11]. Gonadal phenotypes vary from streak gonads, through dysgenetic testes to testes with normal histological structure. The position of the gonad reflects the level of structural differentiation with streak-like gonads being more likely to be found intra-abdominally and well formed testes in the inguinal or scrotal region. Müllerian structures may be present in the most severe cases due to impaired AMH production by Sertoli cells. Gonadal development may be very different between the right or the left side, or even within a single gonad; the more the gonad is affected on one side, the less the müllerian structures are inhibited on the same side (paracrine action of AMH). Somatic features may vary from a Turner syndrome-like appearance (occipital folds, cardiac and renal anomalies) to normal male newborn. Recent amniocentesis data report that up to 90% of fetuses diagnosed as having a 45,X/46,XY mosaic karyotype with the condition confirmed postnatally have a normal male phenotype and apparently normal testis development [12]. Thus, there seems to be limited correlation between the degree of mosaicism on peripheral blood sampling and gonad or somatic phenotype. A number of MGD variants may be present: e.g., 45,X/47,XYY or 45,X/46,XY/47,XYY.

Ovo-Testicular DSD (OT DSD) (46,XX/46,XY chimerism, and variants; old definition true hermaphroditism) has been described in about 500 individuals worldwide and is likely to represent a number of different etiologies. In this syndrome, both ovarian and testicular tissues are present either in the same or in a controlateral gonad. The finding of pure 46,XX/46,XY chimerism is quite rare and has occasionally been described in North American or European patients. By contrast, OT DSD with a 46,XX karyotype is quite common in Africa and the Middle East [13]. The molecular basis of this disorder still needs elucidation; transmission in familial cases reported in the literature is either by autosomal recessive or sex limited autosomal dominant transmission. OT DSD with a 46,XY karyotype is rare and may be explained either by cryptic gonadal mosaicism with a Y chromosome deletion or by an early sex-determining gene mutation [2].

Asymmetry of the gonads and therefore of reproductive ducts and external genitalia is common, with testes, ovaries, and ovotestes present in various combinations. In 50% of unilateral cases, there is an ovotestis on one side and an ovary or testis on the other. In 30% of bilateral cases, testicular and ovarian tissue are present bilaterally, usually as an ovotestis. In 20% of cases, there is a testis on one side, mostly the right, and an ovary on the other side. The ovary tends to maintain its normal anatomical position; the testis, by contrast, may be found anywhere along the pathway of testicular descent, most often in the right inguinal region. Generally, in this disorder, gonadal dysgenesis is less severe than in patients with mixed gonadal dysgenesis [14]. A prevalence of 3–4% of gonadoblastoma and/or germinoma has been estimated in the testicular tissue of patients with 46,XX OT DSD; since the ovotesticular tissue is usually dysgenetic, the removal of this testicular tissue has been recommended [15].

123.4 46,XX DSD

In this section, three different groups of disorders will be described: (1) disorders of ovarian development, (2) disorders of androgen excess, and (3) other conditions affecting sex development.

The first group (ovarian dysgenesis or resistance) are not usually manifest until puberty, when a failure of estrogenization becomes apparent. However, a number of non-metabolic causes of premature ovarian failure (POF) have recently been described (*Xq26-q28*; *Xq22*; *Xq21*; *3q22*; *Xp11.2*; *7q35*; *2p12*; *9q33*), as well as mitochondrial disorders affecting ovarian development (mutant mitochondrial DNA polymerase gamma) [2]. It is possible that more severe forms of these disorders might interfere with earlier aspects of ovarian development and that a prenatal or neonatal ultrasound examination might reveal some anatomic dysgenetic features. Other conditions that can be included in this group, such as 46,XX OT DSD or 46,XX testicular DSD (XX males), have been described previously and there be sufficient testicular tissue in the gonad to produce androgenization of the phenotype and regression of müllerian structures (Table123.3).

The second group of disorders is characterized by androgen excess in 46,XX individuals and congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD), which is the commonest cause of ambiguous genitalia of the newborn [16]. It may be caused less commonly by 11-hydroxylase (11-OHD), 3β-hydroxysteroid dehydrogenase (3β-HSD2) and P450 oxidoreductase (P450-OR) deficiencies (Fig. 123.1, Table 123.4). The clinical presentation is characterized by various degree of genital virilization (Prader staging) with possible genital pigmentation (expression of excessive ACTH

Table 123.3 Genes involved in 46,XX gonadal DSD, with testicular development

Gene / protein type	Chrom. location	Inheritance	Gonad	Müllerian structures	External genitalia	Other associated features
SRY/TF	Yp11.3	Translocation	Testis or ovotestis	–	Male or ambiguous	Infertility
SOX9/TF	17q24	Dup17q24	Not investigated	–	Male or ambiguous	
RSPO1/ sign. molecule	1p34.3	AR	Testis or ovotestis	–	Male	+ Palmoplantar hyperkeratosis and predisposition to squamous cell carcinoma of the skin, +/– congenital bilateral corneal opacities, onychodystrophy, hearing impairment

TF transcription factor.
AR autosomal recessive.
+/– present or absent.

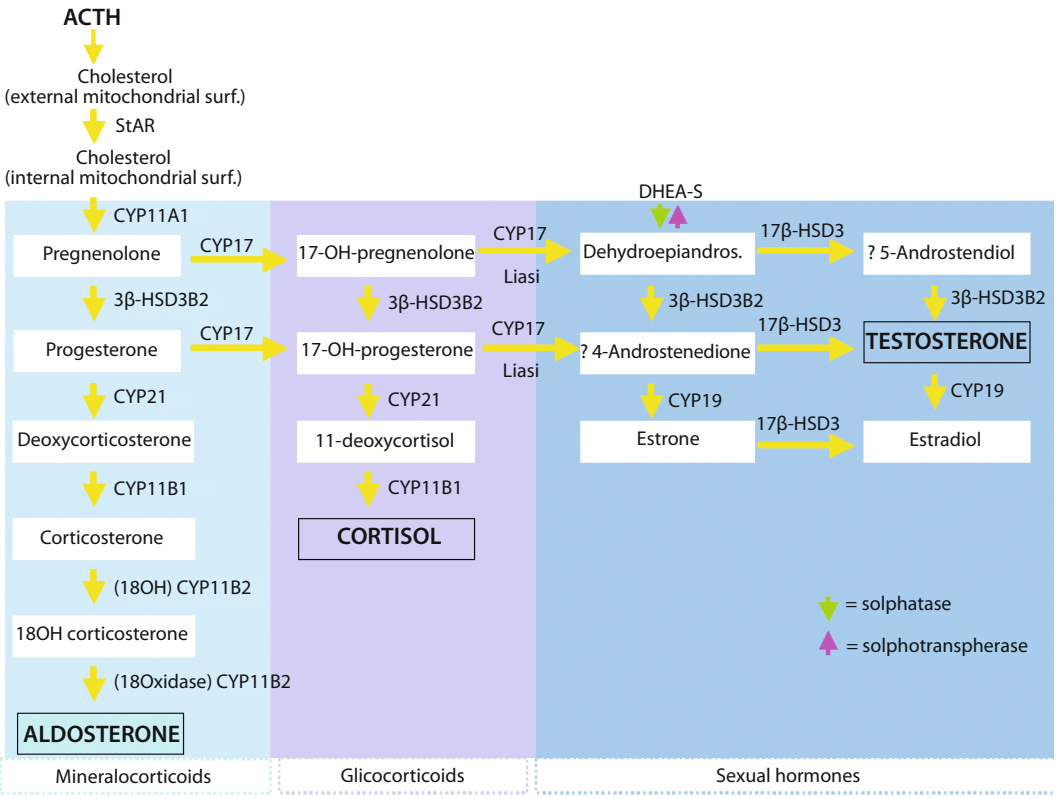

Fig. 123.1 Adrenal and gonadal steroidogenesis

production). A salt losing crisis may be the first presentation in patients with 21-OHD misdiagnosed as males or in the patients with severe 3β-HSD2 and only mild clitoromegaly (due to the peripheral conversion of DHEA to T by 3β-HSD1). 11-OHD can cause marked virilization because of excess testosterone production. Deoxycortocosterone (DOC) is also produced in excess and acts as a mineralocorticoid able to prevent a salt losing crisis, which has been reported as a rare transient manifestation when starting glucocorticoid treatment. Hypertension due to DOC excess is very rare in the neonatal period.

Mutations in the gene encoding the steroidogenetic acute regulatory protein (StAR protein) can also cause CAH, as can P450 oxidoreductase deficiency, which causes mild virilization with biochemical features of both 21-OHD and 17-OHD. Table 123.4 summarizes clinical and hormonal data that are helpful in making a differential diagnosis of the adrenal steroid enzyme defects and lipoid CAH. Diagnosis is based on increased levels of basal and/or ACTH stimulated steroid precursor(s) that accumulate above the enzymatic block, with the exceptions of StAR and P450scc deficiencies in which almost no steroids are produced (Table 123.4).

Table 123.4 Clinical and hormonal data helpful for a differential etiologic diagnosis of the adrenal/gonadal steroid enzyme defects and lipoid CAH

Enzyme deficiency	Sexual ambiguity		Symptoms		Hormonal findings (plasma levels)								
Classical forms	46,XX	46,XY	Salt loss	Hypertension	17-Preg	17OHP	Δ4-A	DHEA	T	Aldo	Renin	11-D	DOC
StAR	No	Yes	Yes	No	↓↓↓↓	↓↓↓↓	↓↓↓↓	↓↓↓↓	↓↓↓↓	↓↓↓↓	↑↑↑	↓↓↓↓	↓↓↓↓
P450 scc[a]	No	Yes	Yes	No	↓↓↓↓	↓↓↓↓	–	↓↓↓↓	↓↓↓↓	↓↓↓↓	↑↑↑	–	–
3β-HSD2	±	Yes	Yes	No	↑↑↑↑	↑	±	↑↑↑	↑↓[b]	↓↓↓	↑↑↑	↓↓	↓↓
P450c21 SW	Yes	No	Yes	No	↑	↑↑↑↑	↑↑↑	N or ↑	↑	↓	↑↑	N	N
P450c21 SV	Yes	No	No	No	↑	↑↑↑	↑↑	N or ↑	↑	N	N (±↑)	N	N
P450c11	Yes	No	No	Yes[c]	N	N or ↑	↑↑↑	↑	↑	↓↓	↓↓	↑↑↑↑	↑↑↑
P450c17	No	Yes	No	Yes[c]	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓	↑↑↑
P450c17 lyase	No	Yes	No	No	N	N	↓↓	↓↓	↓↓	N	N	±	N
P450OR	±	Yes	No	±	↑	↑↑	↑↑	↓↓	↓↓	↓↓	N (±↑)	↓↓	↑↑

[a] Severe forms were thought to be incompatible with survival due to placental failure to produce progesterone. The recent report of several cases demonstrated that severe disruption of P450scc could be compatible with survival in rare instances. Furthermore, heterozygote carriers were healthy and fertile. The possibility of P450scc-independent pathways of steroid synthesis in addition to the current concept of luteo-placental shift of progesterone synthesis in humans has to be questioned.
[b] Normal or elevated for normal girls, or decreased in boys.
[c] Almost never in newborn period.
P450c21 21-hydroxylase, *P450c11* 11-hydroxylase, *3β-HSD* 3beta-hydroxysteroid dehydrogenase, *P450c17* 17-hydroxylase, *P450c17 lyase* 17-20 lyase, *P450 scc* side chain cleavage enzyme, *StAR* steroidogenetic acute regulatory protein, *P450OR* P450 oxidoreductase.
17-Preg 17-OH-pregnenolone, *17OHP* 17-OH-progesterone, *Δ4-A* androstenedione, *DHEA* dehydroepiandrosterone, *T* testosterone, *Aldo* aldosterone, *11-D* 11-deoxycortisol, *DOC* deoxycorticosterone, *N* normal level.

All CAH are genetic disorders with autosomal recessive inheritance. The genes involved in all the defects have been isolated and characterized, and specific mutations have been identified (Table 123.5). Genetic analysis for the identification of mutations of the genes involved is informative for the index case and for future prenatal diagnosis [17]. Fig. 123.2 describes the allelic frequencies of the most common *CYP21A2* gene mutations found in a sample of Italian patients affected by 21-OHD and followed at the Bologna Pediatric-Endocrinologic Centre. This center is able to neonatally screen almost all classical patients of the Emilia-Romagna Region and to prevent salt losing crises of affected males who are most likely to miss early diagnosis [18]. Preterm babies have higher 17-OHP levels than those born at term and specific gestational age and/or weight related data are required [19].

In families at risk for a virilized female baby (because of an index case affected by classical 21-OHD or 11-OHD), prenatal diagnosis and treatment may be undertaken. Early administration (before eight weeks' gestation) of dexamethasone to the mother (20 mcg/kg/pre-pregnancy) prevents genital virilization in most affected females. Treatment has to be started before chorionic villus sampling (CVS) for sex and genetic analyses can be performed at 10–11 weeks' gestation and only female affected fetuses need to be treated throughout

Table 123.5 Genes involved in 46,XX DSD due to androgen excess

Gene/protein type	Chrom. location	Inheritance	Gonad	Müllerian structures	External genitalia	Other associated features
HSD3B2/enzyme	1p13.1	AR	Ovary	+	Female or ambiguous	CAH, primitive adrenal insufficiency, partial androgenization
CYP21A2/enzyme	6p21.23	AR	Ovary	+	Female, ambiguous or male	CAH, +/– adrenal insufficiency
CYP11B1/enzyme	8q21.22	AR	Ovary	+	Female, ambiguous or male	CAH, +/– hypertension
POR/CYP electron donor	7q11.2	AR	Ovary	+	Female or ambiguous	Mixed features of 21-OH Def, 17-OH Def/17-20-lyase Def, aromatase Def; +/– Antley-Bixler S
CYP19/enzyme	15q21	AR	Ovary	+	Ambiguous	Maternal androgenization during pregnancy, absent breast development at puberty, except partial cases

AR autosomal recessive.
+/– present or absent.

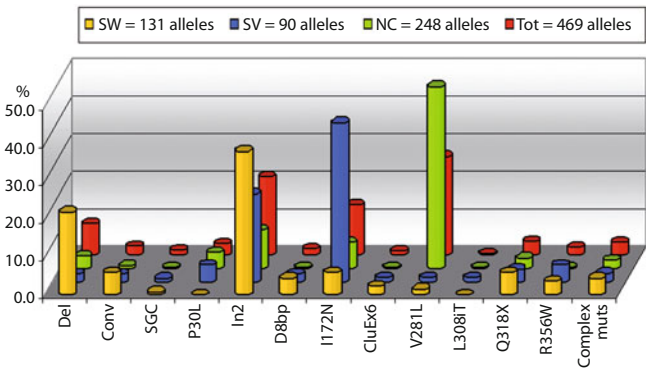

Fig. 123.2 Allelic frequencies of deletions, conversions and mutations present in *CYP21A1P* (469 alleles – Italian Population - Bologna cases)

the pregnancy. To reduce the number of fetuses exposed to dexamethasone unnecessarily, polymerase chain reaction (PCR) testing for cell-free Y DNA in maternal blood at 5–6 weeks of gestation for prenatal sexing is now available as an experimental technique [20, 21]. Due to possible maternal side effects (Cushing syndrome) and lack of long-term trials for its safety, antenatal dexamethasone treatment should be carried out in specialized centers with the use of approved protocols [22].

Aromatase deficiency is a recent, well characterized disorder of steroidogenesis in which there is exposure of the mother and a female fetus to fetal androgens that are normally aromatized by the fetally derived placenta. The lack of aromatase action has a virilizing effect on the mother and on her female newborn [23].

Maternal causes of androgen excess that may virilize a female fetus are either endogenous because of secretion by adrenal and ovarian tumors, or exogenous because of maternal ingestion of androgenic compounds (Danazol).

The third group of disorders refer to some syndromic associations that can cause developmental genital abnormalities in 46,XX girls. Abnormalities of uterine development can result in a bicornuate uterus, uterine hemiagenesis or hypoplasia, or uterine agenesis.

These conditions can be associated with renal, cardiac and cervical spinal abnormalities as part of the Mayer-Rokitansky-Kuster-Hauser syndrome. Uterine anomalies have also been associated with maturity onset diabetes of the young type 5 (*MODY 5*, mutations in *HNF1β*) and with vaginal abnormalities with hand-foot-genital syndrome (mutations in *HOXA13*) and McKusick-Kaufman syndrome (mutations in *BBS6*). Relative clitoral hypertrophy can be associated with conditions such as Frasier syndrome or neurofibromatosis and careful assessment is necessary before a hyperandrogenic cause is diagnosed. Apparent clitoromegaly may be a common finding in premature babies or when there is little labial adipose tissue present: assessment by a physician with experience of normal variability in clitoral size is important.

123.5 46,XY DSD

46,XY DSD are divided into: (1) disorders of gonadal development, (2) disorders of testosterone biosynthesis, metabolism or action, (3) other conditions (Table 123.1).

123.5.1 Disorders of Gonadal Development

Total gonadal agenesis or bilateral gonadal streak (Swyer syndrome) is rare and has been described in 46,XY subjects. There are both internal and external female genitalia, due to the complete absence of testicular determination, with persistent müllerian structures due to insufficient AMH production. The origin of this disorder remains uncertain but may be due to a defect in genes critical to bipotential gonad development [24].

Partial gonadal dysgenesis has been reported in some clinical conditions, characterized by impaired testicular development, ambiguous external genitalia with or without müllerian structures (similarly to 45,X0/46,XY conditions). Mutations in the *SRY* gene have been related to this phenotype in about 20% cases (mostly as de novo mutations). Heterozygous mutations in SF1 gene have also been recently found to be causative of XY DSD without apparent adrenal involvement [25].

In ovotesticular DSD, which is a very rare disorder, the gonad has both ovarian and testicular tissue. The karyotype is usually not 46,XY but a mosaic.

Testicular regression syndrome is a condition in which a testis is thought to have once existed but has atrophied and disappeared during early development. The timing of the regression process is critical. If this process occurred before differentiation of the male external genitalia is completed, the newborn will have an undervirilized appearance (DSD), while if it happens later in gestation, the male newborn will have cryptorchidism with undetectable testis (vanishing testis).

Genetic mutations and chromosomal changes involved primarily in 46,XY gonadal DSD may be associated with other clinical features (Tables 123.6, 123.7).

123.5.2 Disorders of Testosterone Biosynthesis

During intrauterine male development, both maternal hCG and fetal LH act on Leydig cells by a hCG/LH common-G-protein coupled receptor, stimulating testosterone production. A mutation in this receptor gene may cause Leydig cell hypoplasia with complete or partial impairment of hormone secretion, so that an undervirilized variable phenotype arises at birth [26].

Testosterone biosynthesis, like all steroidal hormones, starts from cholesterol as precursor.

7-Dehydrocholesterol reductase (DHCR7) deficiency is a disorder affecting the cholesterol biosynthesis pathway and

Table 123.6 Genes involved in 46,XY gonadal DSD

Gene/protein type	Chrom. location	Inheritance	Gonad	Müllerian structures	External genitalia	Other associated features
SRY/TF	Yp11.3	Y	Dysgenetic testis or ovotestis	+/–	Female or ambiguous	–
SOX9/TF	17q24-25	AD	Dysgenetic testis or ovotestis	+/–	Female or ambiguous	Camptomelic dysplasia
WT1/TF	11p13	AD	Dysgenetic testis	+/–	Female or ambiguous	Wilms' tumor, nephropathies, gonadoblastoma (WAGR, Denys-Drash and Frasier syndromes)
SF1 (NR5A1)/ TF (NR)	9q33	AD/AR	Dysgenetic testis	+/–	Female or ambiguous	+/– Primitive adrenal insufficiency
DHH/sign. molecule	12q13.1	AD/AR	Dysgenetic testis	+	Female	+/– Minifascicular neuropathy (1 case)
ATRX/Helicase	Xq13.3	X	Dysgenetic testis	–	Female, ambiguous or male	α-Thalassemia, mental retardation
ARX/TF	Xp22.13	X	Dysgenetic testis	–	Ambiguous	+ Lissencephaly, epilepsy, temperature instability

TF transcription factor, *NR* nuclear receptor, +/– present or absent.
AD autosomal dominant (or de novo mutation), *AR* autosomal recessive, *Y* Y-linked, *X* X-linked.

Table 123.7 Chromosomal changes involved in 46,XY gonadal DSD

Candidate gene/ protein type	Chrom. location	Inheritance	Gonad	Müllerian structures	External genitalia	Other associated features
DAX1 (NROB1)/ TF (NR)	Xp21	Dupl Xp21	Dysgenetic testis or ovary	+/–	Female or ambiguous	
DMRTs/TF	9p24.3	Del 9p24.3 red. penetrance	Dysgenetic testis or normal testis	+/–	Female or ambiguous	Mental retardation, somatic dismorphisms
WNT4/sign. molecule	1p35	Dupl 1p35	Dysgenetic testis	+	Ambiguous	Mental retardation

TF transcription factor, *NR* nuclear receptor.
+/– present or absent.

causes Smith-Lemli-Opitz syndrome (which includes microcephaly, mental retardation, facial dysmorphism, polydactyly genital anomalies; renal hypoplasia and unilobar lung are also commonly associated).

Lipoid adrenal hyperplasia due to deficiency of StAR protein, which facilitates the rapid movement of cholesterol from the outer to the inner mitochondrial membrane, is rare in Europe and America, but more common in Japan. Patients are phenotypic females (despite having a testis) with enlarged adrenal cortex, engorged by cholesterol and cholesterol esters. Severe adrenal insufficiency is the main potentially lethal consequence, since virtually no steroids are made [27].

Enzymatic defects altering the normal testosterone bio-synthesis, cholesterol side chain cleavage (*CYP11A1*), 3β-hydroxysteroid dehydrogenase (*3β-HSD2*), 17α-hydroxylase/17-20-lyase (*CYP17*), and P450 oxidoreductase (POR) enzyme deficiencies, are inherited as autosomal recessive conditions. The clinical presentation of this group of DSD is characterized by various degree of genital under-masculinization with possible genital pigmentation due to excessive ACTH production in cases associated with antenatal insufficiency. A salt losing crisis and hypoglycemia may be the first clinical sign. Babies with severe deficiency are often misdiagnosed as females.

P450scc (*CYP11A1*) is the mitochondrial enzyme that converts cholesterol to pregnenolone and is therefore responsible for the first rate-limiting step in steroid synthesis, which is required for pregnenolone production by the placenta as well as for mineralcorticoid, glucocorticoid and androgen production by the adrenal glands and gonads.

P450c17 or *CYP17* (gene locus in 10q24.3) are expressed both in the zona fasciculata and in the zona reticularis of the adrenal cortex and in gonadal tissues as a steroidogenic enzyme with dual functions, hydroxylation and as a lyase. The first activity results in hydroxylation of pregnenolone and progesterone at the C17 position to generate 17α-hydroxypregnenolone and 17α-hydroxyprogesterone. The latter enzyme activity cleaves the C17–C20 bond of 17α-hydroxypregnenolone and 17α-hydroxyprogesterone to form dehydroepiandrosterone and androstenedione respectively.

Patients with *3β-HSD2* usually show some degree of virilization (due to peripheral conversion of DHEA to T by 3β-HSD1) or hypospadias, which suggests the diagnosis. In

17-OHD deficiency the genital phenotype may vary from that of a complete female to severe hypospadias; corticosterone (C) and deoxycortocosterone (DOC) are produced in excess and have glucocorticoid and mineralocorticoid functions, respectively. Hypertension due to DOC excess is very rare during the neonatal period. In 17-20 lyase deficiency, the genital phenotype is similar, but there is no cortisol deficiency and no mineralocorticoid excess.

As in 46,XX patients, P450 oxidoreductase deficiency may cause mild virilization of a male fetus, with the biochemical features of both 21-OHD and 17-OHD [28]. Table 123.4 summarizes the clinical and hormonal data for the differential etiologic diagnosis of adrenal steroid enzyme defects and lipoid CAH. For the enzymatic diagnosis see discussion of congenital adrenal hyperplasia in § 123.4 on 46,XX DSD.

46,XY DSD due to mutations in the *HSD17B3* gene (encoding the 17β-HSD3 isoenzyme) is characterized by female-like or ambiguous genitalia at birth, with the presence of a blind vaginal pouch, intra-abdominal or inguinal testes. Impaired conversion of androstenedione into testosterone explains the virilization defect. Since virilization occurs at puberty, most affected males have been raised as females and changes to male gender role behavior at puberty have been reported [29].

123.5.3 Disorders in Testosterone Metabolism: 5-Alpha-Reductase Deficiency

SRD5A2 gene (2p23) encodes the steroid 5-alpha-reductase 2 isoenzyme, which converts testosterone into dihydrotestosterone (DHT) mainly in target tissues.

Affected 46,XY individuals have normal or increased testosterone plasmatic levels, with decreased DHT levels and an elevated testosterone/DHT ratio in a basal blood sample on the 1st and 2nd days and at 1–3 months of age. Newborns present with ambiguous undervirilized or almost female external genitalia. However, wolffian differentiation occurs normally and they have epididymides, vas deferens and seminal vesicles. The testes are often located in the abdomen or in the inguinal region, suggesting that DHT influences gonadal migration in the scrotum. Virilization occurs at puberty and some patients, who were not submitted to orchidectomy in childhood, have undergone a male social sex change (Table 123.8). In adulthood, the prostate is small and rudimentary, the penis is small and facial and body hair is absent or decreased and balding has not been reported. Spontaneous fertility has been reported when orchidopexy has been properly performed, so that male gender should be assigned. Correct early diagnosis is mandatory.

The main differential diagnoses are described in Table 123.1 and molecular analysis of the *SRD5A2* gene is indicated during the newborn period before assigning female sex and performing gonadectomy [30, 31].

123.5.4 Disorders in Testosterone Action

An androgen receptor (AR) mediates the pleiotropic effects of testosterone. The phenotype of androgen insensitivity, an X-linked recessive disorder, depends on the degree of AR disruption. The phenotype of complete androgen insensitivity syndrome (CAIS) is that of a normal female, despite the presence of an XY karyotype. The testis is histologically normal and produces testosterone concentrations within the age-appropriate normal male range. Data on prevalence are imprecise, ranging from 1:20,400 to 1:99,000 genetic males.

Presentation may vary from the perinatal period to adulthood. In pregnancy, the diagnosis may be suspected because of a male fetal karyotype and female phenotype at antenatal ultrasound or at birth. During the perinatal period and childhood, the main presentation of CAIS is as a bilateral inguinal or labial hernia. All girls with this type of hernia should therefore be investigated for CAIS.

In partial androgen insensitivity syndrome (PAIS), the external genitalia may be ambiguous at birth, thus making immediate sex assignment difficult. The phenotype varies, depending on the degree of hormonal resistance, from perineoscrotal hypospadias, micropenis and a bifid scrotum with undescended testes, to isolated clitoridomegaly, marginally different from CAIS. The milder end of the spectrum of PAIS includes isolated hypospadias in the newborn and infertility in adulthood (minimal androgen insensitivity syndrome [MAIS]).

PAIS should be differentiated from sex chromosome DSD with 45,X0/46,XY or 46,XX/46,XY karyotype, defects in gonadal development or a defect in androgen biosynthesis. Dynamic endocrine investigations (hCG stimulating test and/or tests to assess androgen sensitivity) should be performed to select patients for genetic investigation. A number of patients, previously misclassified as PAIS without demonstrated AR mutations, have been recently diagnosed as heterozygous carriers of SF1 (NR5A1) mutations. In the majority of these cases the adrenal function was apparently normal [25].

123.5.5 Persistent Müllerian Duct Syndrome

Anti-müllerian hormone (AMH), a member of the transforming growth factor beta (TGF-beta) family, is produced by gonadal somatic cells and is mainly responsible for the regression of müllerian ducts during male sex differentiation with signaling through two serine/threonine kinase receptors. A defect of AMH causes the persistence of müllerian derivates in otherwise normally virilized males. Its inheritance is as a recessive autosomal and is due, in 84% of cases, to mutations of *AMH* and *AMH* receptor type II genes. Serum AMH is normal for age in patients with *AMH* type II mutations and low or undetectable in those with AMH mutations (although some exceptions have been recently described [32]). In 14% of cases the origin of the condition is unknown [33].

Table 123.8 Comparison of main clinical and laboratory findings among Leydig cell hypoplasia/aplasia, 17β-HSD3 deficiency, 5α-reductase deficiency and complete androgen insensitivity syndrome (AIS) [29]

	Leydig cell hypoplasia	17β-HSD3 deficiency	5α–Reductase deficiency	Complete AIS
Eponymous	–	17-Ketosteroid reductase deficiency	Pesudovaginal perinoscrotal hypoplasia	Testicular feminization syndrome
Karyotype	46,XY	46,XY	46,XY	46,XY
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive	X-linked recessive
External phenotype	Female or ambiguous	Female or ambiguous	Ambiguous or female	Female
Blind ending vagina	Present (70-80%)	Present (80%)	Present (50%)	Present (100%)
Internal phenotype				
Wolffian structures	Male (hypoplasic)	Male	Male	Absent or male (hypoplasic)
Prostate	–	Absent/ highly hypoplasic	Absent or hypoplasic	Absent
Müllerian structures	Absent	Absent	Absent	Absent or rudimental (~30%)
Testes	Intra- or extra-abdominal	Extra-abdominal	Extra-abdominal	Intra-abdominal
(% of patients)	–	(~90%)	(~100%)	(70%)
Hormone profile	↓↓ All androgens T/DHT N, Estrogens N or ↓	Δ^4-A ↑; T ↓, T/ Δ^4-A ↓ T/DHT ↑,T/ Δ4-A N, Estrogens N	Δ^4-A N; T N or ↑, DHT ↓, T/DHT N or ↓, Estrogens ↑	Δ^4-A N, T N or ↑, T/ Δ^4-A N
Gender assignment at birth	Female or ambiguous	Mainly female	Female or male	Female
Puberty	Sexual infantilism/virilization	Virilization	Virilization	Feminization
breast development	Absent	Variable	Absent	Normal female
androgen hair	Variable	Normal male	Normal male	Absent or scanty
Gender role change	Not present	Present	Present	Not present
(% of patients)	(30-50%)*	(~75%)**		
Brain androgenization	Dependent on impairment of androgen production	Dependent on prenatal and postanatal T production	Likely normal	Absent
Genetics, mutation of	*LHGCR*	*17β-HSD3* gene	*SRD5A2*	*AR* gene
Chromosomal gene map	2p21	9q22	2p23	Xq11-12

* Mainly in Israel and Eastern countries; **mainly in the areas with high prevalence, but also in Western countries.
N normal, ↑ increased, ↓ decreased, Δ^4-A Δ^4-Androstenedione, *T* testosterone, *DHT* dihydrotestosterone.

123.5.6 Endocrine Disruptors in Pregnancy

Timing of hormone secretion is critical for normal organogenesis, and any external disruption is therefore time-dependent. Androgen action is most critical since it causes masculinization of the external genitalia early in pregnancy (from week 7–8). Androgen exposure of the female fetus during this period will cause masculinization, whereas impaired androgen action in a male fetus will result in under-masculinization [34].

Endocrine disruptors with androgenic or antiandrogenic potency or estrogenic chemicals (i.e., diethylstilbestrol) may therefore interfere with sexual differentiation. Hypospadias and cryptorchidism can be caused by antiandrogens. Phthalate esters, ubiquitous in the past and still widely used as plasticizers, disturb androgen biosynthesis and exposure to these chemicals causing antiandrogenic effects has been related to inhibition of Leydig cell function [35]. Exposure to phthalates occurs via skin, airways, intravenous transfusion, food, and drink [36] and absorption through the skin is very rapid [37].

The first step to prevent environmental causes of disorders of sexual differentiation is the effective recognition of endocrine disrupting properties of chemicals on developing fetuses and children. However, although great effort as been put into the development of robust testing methods of endocrine disruptors in industrialized countries, progress is slow.

123.6 Ethics and Sex Assignment

Initial management of DSD depends on establishing an early diagnosis. A precise diagnosis may, however, be difficult in many cases of 46,XY DSD and in more than 50% of the cases no conclusive diagnosis is made. Nevertheless, there are also legal reasons for assigning a gender to all newborns with ambiguous genitalia, even though it is possible to postpone assignment when expert assessment is needed. At this critical early stage, discussion with the parents must be open, and parents' participation in decision-making should be encouraged.

A multidisciplinary team should be established at the time of the the first communication of the diagnosis of DSD with the aim of helping professionals and parents to evaluate the pros and the cons of all medical and surgical options. The multidisciplinary team should help with anxieties both in physicians and parents that may lead to premature and irreversible decisions [38–40]. According to the 5th World Congress on Family Law and Children Rights (Halifax, August 2009), the principles of ethical guidelines for the management of infants with DSD should be: 1) minimising physical risk to child, 2) minimising psychosocial risk to child, 3) preserving potential for fertility, 4) preserving or promoting capacity to have satisfying sexual relations, 5) leaving options open for the future if necessary, and 6) respecting the parents' wishes and beliefs if it is possible [41].

There is general agreement that 46,XY DSD due to complete testicular dysgenesis, severe testosterone biosynthesis defect or Leydig cell aplasia should be assigned to the female sex.

In CAIS, gender assignment and sex of rearing is female. Inguinal hernias need repair. There is no uniformity of practice for early versus late gonadectomy: parents require appropriate information to enable them to reach a joint decision with the professional health care team. Two important points for discussion are the merits of spontaneous puberty with gonads in situ versus induction of puberty by oestrogen replacement therapy, and the possible risk of gonadal tumor development (usually seminoma) if gonadectomy is delayed until adulthood.

In 46,XX CAH with genital virilization, gender assignment is female, even when there is complete virilization at birth. Steroid replacement is mandatory. In salt-wasting forms, it is necessary to start this therapy as soon as possible in order to prevent electrolytes imbalance or to treat hypovolemic shock.

In 5-α-reductase deficiency, male assignment is the first choice. Possible assignment to the male sex should be seriously discussed in cases of 17-β-OH-steroid-dehydrogenase deficiency and 46,XY DSD due to PAIS or partial testicular dysgenesis. Many authors suggest that gender assignment should also be based on prenatal androgen brain exposure [42, 43].

Social and cultural factors, as well as hormonal effects, appear to influence gender role and gender role change. DSD may carry a stigma. In some societies, female infertility precludes marriage, which also affects employment prospects and creates economic dependence. Religious and philosophical views may influence how parents respond to the birth of an infant affected by this medical condition. There may also be fatalism and guilt feelings related to congenital malformations or genetic conditions, and poverty and illiteracy impair access to health care [44]. Support groups integrate the work of the health care team. Peer and parent support groups may provide a context in which intimate issues of concern can be approached safely with someone who has been there, even though parents may be more amenable to these discussions than adolescent patients. Support groups can also help families and consumers to find the best quality care (www.dsd.guidelines.org).

Long-term outcome should be studied in every treatment center, paying special attention to different cultural settings [43, 45]. So far, studies have shown that while many patients fare well and are leading productive lives, gender dysphoria has been underestimated in the past and gender counseling as well as sexual counseling should be part of the multi-disciplinary service concerning DSD patients. More emphasis is also needed on strategies to prevent the development of germ cell cancers (i.e., seminoma in CAIS and dysgerminoma in gonadal dysgenesis DSD) or unnecessary gonadectomy. Urological problems in both males and females with DSD should not be underestimated [46].

In cases of genetic defects carried by both parents as heterozygous, genetic counseling should be planned and offered to families (risk of recurrence).

123.7 Medical Management of DSD in the Newborn

The evaluation of a newborn with abnormalities of genitalia should be carried out by an experienced multidisciplinary team. A careful history should take account of any family history of unexplained neonatal death, siblings with virilization or precocious puberty or female sexual infantilism, maternal drug assumption or androgen changes during pregnancy. There should also be a complete physical examination (external genitalia appearance including the presence or absence of palpable gonads, measurement of phallus length, urethral opening identification, presence or absence of a vagina), laboratory and radiological investigations.

A diagnostic schedule is proposed in Fig. 123.3. At 24–48 hours and 72–96 hours of life, blood samples should be collected and a serum sample should be stored to decide on the most appropriate diagnostic test after the results of karyotyping.

Timing is important because of the physiological changes which occur in the hormonal profile shortly after birth [47]. If hormonal tests (i.e., hCG stimulation test) and imaging investigations do not lead to a definite diagnosis, laparoscopy should be performed. During surgery, skin biopsy may be used for functional studies on intracellular mechanisms induced by androgen receptor activation.

The standard treatment of CAH is hydrocortisone 10–20 mg/m^2/day (3.0–5.0 mg/day) usually in three divided doses (10 mg tablets can be dissolved in 2 mL water). For sick infants, 2 mg/kg of hydrocortisone hemisuccinate by i.v. bolus, followed by 20–30 mg/m^2/day by constant i.v. infusion may be a more appropriate initial treatment. Blood electrolytes should be monitored daily from day 3 and if K^+ raises or Na^+ falls (salt loss may occur between 6 and 21 days of life) 9α-fludrocortisone should be started at 0.05 mg/day in two divided doses. The latter dose may be increased to 0.2 mg/day in two divided doses in resistant cases. Salt supplementation (5 mEq/kg/day; 5 mL sol 20% NaCl =1 g = 17 mEq) complements mineralocorticoid treatment during the first 6 months of life. For the management of acute adrenal insufficiency, see § 123.4 on 46,XX DSD.

123.8 Surgical Management of DSD: an Approach

There is an increasing tendency to recommend that any decision about genital surgery should be made by the parents and, when appropriate, the patient, in discussion with the medical team [48]. Of course, patient involvement in decision making, implies the delay of all the interventions not primarily directed to functional repair or to remove malignant tissue.

The goal is to construct genitalia that are compatible with the assigned gender sex, prevent urinary infections or incontinence, and provide good adult sexual and reproductive

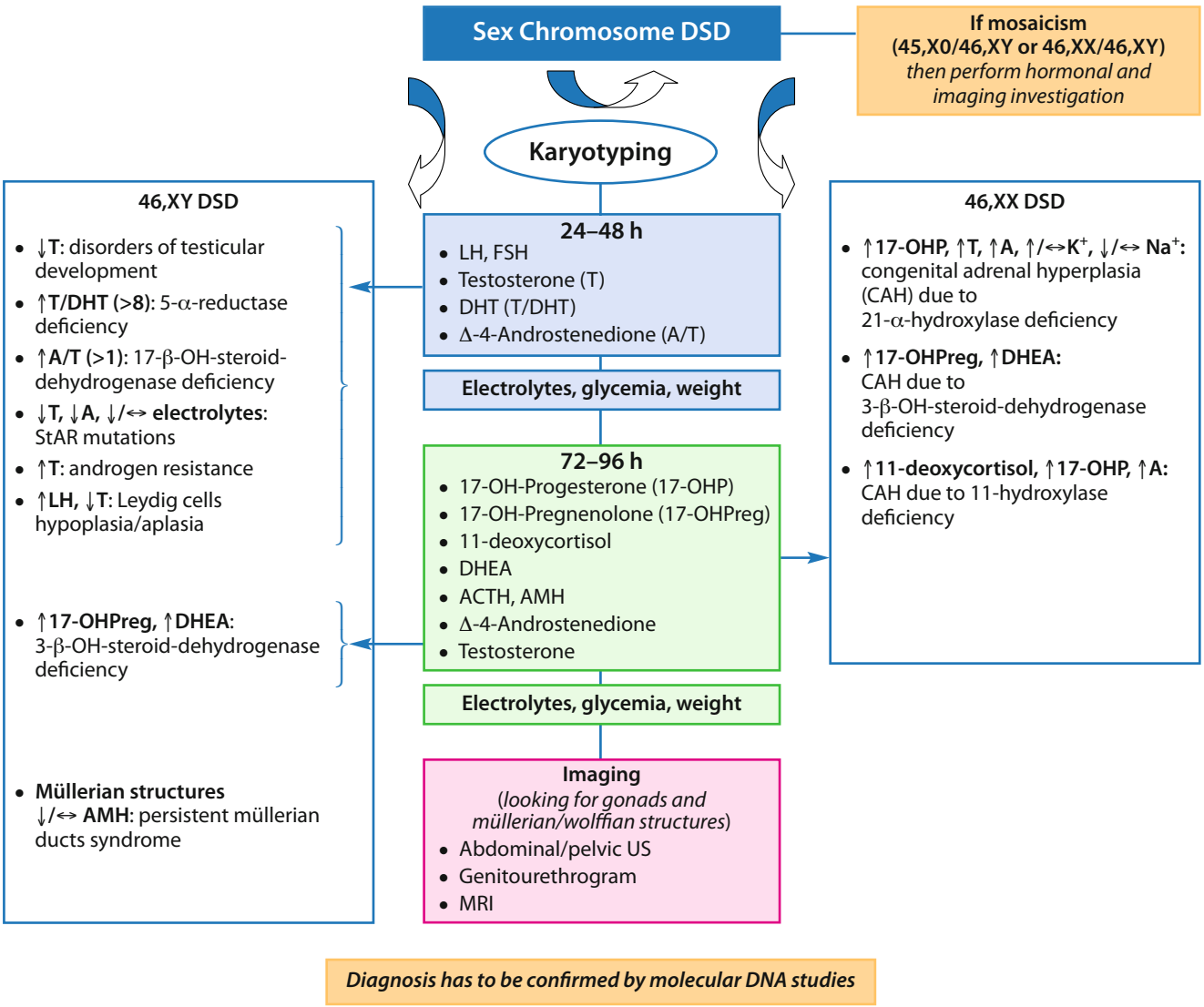

Fig. 123.3 A diagnostic schedule for early diagnosis of DSD in the newborn

function [49]. Surgeons with expertise in DSD management, together with the DSD team, need to explain the surgical sequences and the transition from infancy to adulthood.

There is controversy about the timing of feminizing genitoplasty (FG) in girls with virilising CAH. Many pediatric urologists and endocrinologists continue to recommend early FG in agreement with the guidelines of American Academy of Pediatrics (recommended period between 2 and 6 months of age). However, intersex advocacy groups would delay genital surgery. When possible, FG should be a one stage repair. Clitoroplasty should only be considered in cases of severe virilization (Prader III–V) with emphasis on functional outcome rather than cosmetic appearance. Vaginoplasty should be performed in infancy only if the persistence of a urogenital sinus causes complications. Generally, surgical refinement needs to be done at puberty. Vaginal dilation is not recommended during childhood, while its use during young adulthood may be useful in avoiding the need for bowel vaginoplasty, especially in women with CAH or CAIS.

In patients raised as males, the repair of hypospadias with correction of chordee, urethral construction and orchidopexy for undescended testes are recommended. Because of limitations in penile reconstruction, the complexity of phalloplasty should be explained to the parents during initial counseling, especially if gender assignment depends on it.

The risk of gonadal malignancy should inform the timing of gonadectomy, although there is some controversy about this. Testes in individuals with 46,XY gonadal dysgenesis or fragments of Y-chromosome material raised as female should be removed early if there is no possibility of realistically controlling gonadal development. Among those with gonadal dysgenesis and a scrotal testis, the current

recommendation is to perform testicular biopsy at puberty looking for malignancy.

For female patients with ovotesticular DSD and functional ovarian tissue, separation and removal of the testicular component in early life is advised in an attempt to preserve fertility. Gonadectomy should be performed before puberty in PAIS, 17β-HSD, and 5α RD deficiencies and in 45,X/46,XY mixed gonadal dysgenesis raised as females.

123.9 Cryptorchidism and Hypospadias

Cryptorchidism, failure of the testis to descend from its intra-abdominal location into the scrotum, has a prevalence of approximately 3% in newborns at term, but is 10 times higher in preterms. If there is late spontaneous descent during the first 6–9 months of life, the prevalence of cryptochidism declines to 1% in infants born at term but it is still three fold higher in infants born preterm or low birth weight [50].

Using a two-handed technique, testes may be palpable (inguinal or prescrotal) or impalpable (abdominal). If cryptorchidism is unilateral, the consistency and size of the undescended testis should be noted, in comparison with the opposite one.

A family history should be investigated for cases where there is doubt about the diagnosis of DSD and for other cases of undescended testes. If cryptorchidism is bilateral (impalpable testes in an otherwise normal boy), urgent karyotype is mandatory as well as in cases of cryptorchidism associated with an ambiguous appearance of external genitalia (see Medical Management).

The testosterone response to hCG stimulation can clarify normal Leydig cell function, and elevated gonadotropin levels suggest primary gonadal failure. The evaluation of AMH and inhibin B levels, secreted by Sertoli cells, may be a sensitive marker for the presence of functioning testicular tissue. Imaging (ultrasound [US], magnetic resonance imaging [MRI]) may be useful for identifying inguinal or abdominal testes, but there is a high false-negative rate.

If one or both testes are palpable and the genitalia are normal male, the newborn should be referred to a pediatric surgeon. Hormonal treatment (intramuscular hCG or intranasal GnRH) was largely used in the past, but there are many different protocols. Hormonal therapy induced permanent testicular descent in a minority of young cryptorchid boys with either unilateral or bilateral palpable testis [51, 52].

These days, surgical treatment is preferred and orchidopexy is performed during the 1–2nd year of life, both to preserve fertility and to monitor testicular cancer lesions (20–48 fold increased risk in undescended testes; the incidence of malignant transformation related to testicular dysgenesis syndrome is also increased in the unaffected testis [53, 54].

Hypospadias is a congenital displacement of the urethral meatus in male newborns. The exact position of the meatus determines whether the form of hypospadias is glandular or coronal (mild), penile (moderate), scrotal or perineal (severe). While most cases of mild forms usually occur as isolated defects, severe forms may be a sign of DSD. The prevalence is about 3–4 per 1000 live births. The pathogenesis of isolated hypospadias is multifactorial (genetic, endocrine and environmental factors) [55]: however, the prevalenceof hypospadias is higher in infants born small for gestational age than in newborns of normal birth weight.

Patients need surgical repair, since they may have urological disorders and subfertility [56].

Cryptorchidism and hypospadias may be part of the clinical spectrum of complex genetic syndromes, e.g., Opitz GBB syndrome, Robinow syndrome and CHARGE association.

Opitz syndrome (22q11.2 del) is marked by hypertelorism or telecanthus, laryngotracheoesophageal cleft, clefts of lip, palate, and uvula, swallowing difficulty and hoarse cry, genitourinary defects (hypospadias in males and splayed labia majora in females), mental retardation; developmental delay, congenital heart defects.

Robinow syndrome (autosomal dominant or autosomal recessive due to a mutation of receptor tyrosine kinase-like orphan receptor gene-2, *ROR2* in 9q22) may present with mesomelic limb shortening, facial and genital anomalies.

The CHARGE association, possibly due to a mutation of 7q21.11 (semaphorin-3E gene, *SEMA3E*) or 8q12.1 (chromodomain helicase DNA-binding protein-7, CHD7), is characterized by coloboma of the eye, heart anomalies, choanal atresia, delayed mental and somatic development, microphallus, ear abnormalities and/or deafness. Facial palsy, cleft palate, and dysphagia are commonly associated.

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124

Pathophysiology of Fetal and Neonatal Kidneys

Farid Boubred, Isabelle Grandvuillemin and Umberto Simeoni

124.1 Kidney Development

The definitive kidney, the methanephros, is formed by two processes, nephrogenesis (the formation of glomerulus and tubules) and branching morphogenesis (the formation of collecting ducts, calyces, pelvis and ureters). The metanephric kidney takes place after the formation and involution of two embryonic kidneys, the pronephros (non functional organ) and the mesonephros (first functional kidney), that in turn evolves into the ureteric bud (UB). The metanephros appears during the fifth gestational week, and develops from the specific interaction between the epithelial ureteric bud and the undifferentiated metanephric mesenchyme (MM). This interaction is crucial for the differentiation of the mesenchyme and the induction of UB branching division (Fig. 124.1). The UB arises in response to signals elaborated by the mesenchyme and then undergoes branching morphogenesis following the invasion of the MM by the UB. This process gives a 15 branch generation. At 20–22 weeks of gestation, branching morphogenesis is completed and results in the collecting system. Mesenchymal cells that are in close contact with the invading UB undergo an epithelial transformation. The induced metanephric mesenchyme (MM) gives the nephrons, through the consecutive stages of condensation, renal vesicule, vascular cleft, and S-shaped body. The glomerular capillary tuft is formed via recruitment and proliferation of endothelial and mesangial cells precursors. The nephrons develop in successive stages from the inner to the outer area of the fetal kidney in parallel with the vascular system. In the human, the primitive glomerulus appears at approximately 9–10 weeks of gestation. The nephrogenesis is completed by 34–36th weeks of gestation [1]. About 60% of the nephrons develop during the third trimester of gestation, while nephrogenesis may continue ex-utero in preterm infants [2]. Once nephrogenesis is complete, stroma cells differentiate into fibroblasts, pericytes and lymphocytes-like cells. At birth the final nephron number varies from 800,000 and 1 million per kidney. Such variation in nephron number is due to genetic factors and to the fetal environment [3]. Several genes and molecular pathways control the formation of the renal collecting system and nephrogenesis, such as transcriptional factors (PAX-2, WT1), growth factors (IGF, EGF, TGF), oncogenes, the extracellular matrix, and vascular factors (Table 124.1) [4]. These factors act at a specific time of kidney development especially when the UB interacts with the adjacent MM. Blockade of vascular endothelial growth factor receptor (VEGF-R), inhibition of the

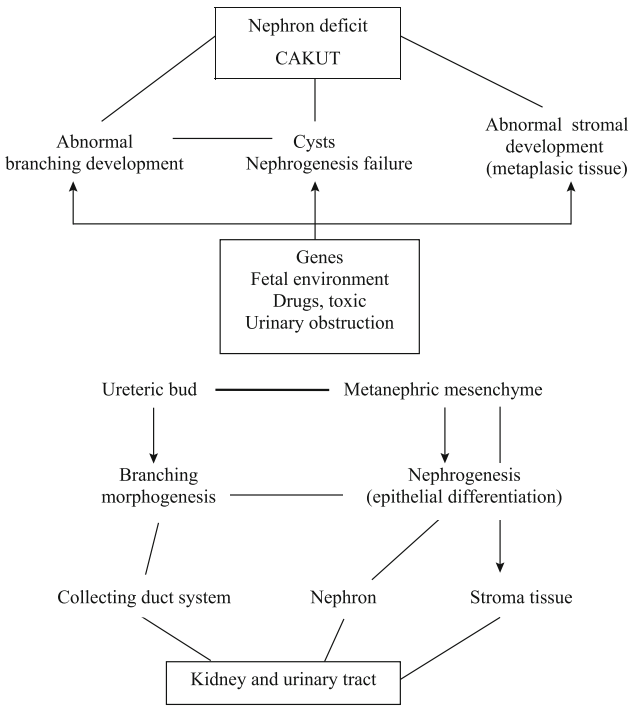

Fig. 124.1 Kidney development: simplified schematic process of normal and pathologic kidney development

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 124.1 Syndromes and gene defects associated with abnormal kidney development (CAKUT)

Syndrome	Gene defects	Kidney malformations
Renal-coloboma syndrome	*PAX 2*	Hypoplasia, VUR
Renal cysts and diabetes syndrome	*HNF1b*	Dysplasia, hypoplasia
Branchio-oto-renal syndrome	*EYA1, SIX1*	Unilateral or bilateral agenesia/dysplasia
Renal tubular dysgenesis	*RAS components*	Tubular dysplasia/dysgenesis
Campomelic dyslasia	*SOX9*	Dysplasia, hydronephrosis
Townes-Brock syndrome	*SALL1*	Dysplasia, hypoplasia, VUR
Simpson-Golabi-Behmel syndrome	*GPC3*	Medullary dysplasia
Kallman syndrome	*KAL1, FGFR1, PROK*	Renal agenesis
Fraser syndrome	*FRAS1*	Dysplasia, renal agenesis
Alpert syndrome	*FGRG2*	Hydronephrosis
Alagille syndrome	*JAGGED1*	Cystic dysplasia
Meckel-Gruber syndrome	*MKS1, MKS3*	Cystic renal dysplasia
Hypoparathyroidism, deafness and renal anomalies syndrome (HDR)	*GATA3*	Dysplasia, VUR
Di George syndrome	*22q11*	Agenesia, dysplasia , VUR
Beckwith-Widemann syndrome	*P57*	Dysplasia
Pallister-Hall syndrome	*GLI3*	Dysplasia
Nail-patella syndrome	*LMX1B*	Agenesia, glomerular anomalies
Smith-Lemli-Opitz syndrome	*7 hydroxy-cholesterol reductase*	Agenesia, dysplasia
Zellweger syndrome	*PEX1*	Cystic dysplasia, VUR
Glutaric aciduria type II	*Glutaryl CoA dehydrogenase*	Cystic dysplasia

renin angiotensin system (RAS) and knock-out for cyclooxigenase (COX)-2 gene expression are associated with impaired nephrogenesis including glomerular cysts, dysplasic tubules and tubular dysgenesis [5–7].

124.2 Renal Physiology

124.2.1 The Fetus

During intrauterine life, the homeostasis of the fetus is assigned to the placenta. Glomerular filtration rate, renal blood flow and tubular functions progress with renal growth and nephrogenesis. The kidney is involved in urine production, which is essential in fetal well-being, and in hormonal production (1,25 OH vitD_3 and erythropoietin). In the near-term period, the fetal kidney shows sufficient glomerular and tubular development to allow the adaptation to extrauterine life [8].

124.2.1.1 Glomerular Function and Renal Blood Flow

During fetal life, nephrogenesis plays an important role in maturation of the glomerular filtration rate (GFR). GFR in the fetus is low, even at the end of gestation, and depends on various factors especially on renal blood flow.

In the fetus, the systemic arterial blood pressure, around 40–60 mmHg, and renal blood flow are low in comparison with the newborn or the adult. In the sheep, the fetal kidneys receive 3% of cardiac output compared to 15% in the neonatal period. Such low renal blood flow (RBF) is related to elevated renal vascular resistances. This state is mainly due to a subtle equilibrium between vasoconstrictive factors, including the RAS and the renal sympathic nervous system, and vasorelaxing factors such as the prostaglandins, nitric oxide, and other factors (Fig. 124.2). In the fetal kidney, the RAS is up-regulated. The fetus is able to release renin into the fetal circulation. Renin does not cross the placenta and the fetal plasma renin levels are higher than maternal levels. Angiotensin II increases mainly the glomerular efferent tone, which is highly sensitive to angiotensin. Up-regulation of the RAS maintains renal blood flow and renal perfusion pressure.

The renal sympathetic nervous system increases renal vascular tone in afferent and efferent arterioles. The fetal renal vasculature is more sensitive to alpha-2 adrenoreceptor stimulation than the neonatal one. Up-regulation of alpha-2 receptors is associated with a down-regulation of beta-2 adrenoreceptors. Adenosine vasodilates glomerular arterioles in the physiologic state, but reduces GFR in stress conditions (increasing tone in afferent glomerular arteriole).

Vasoconstrictive forces are counterbalanced by vasodilating factors that act on the glomerular afferent arteriole to maintain a sufficient renal blood flow. Theses factors are mainly prostaglandins, nitric oxide (NO), and the kallikrein-kinin system. Prostaglandins, in particular prostanglandins E2 and I2, are of major importance. These prostaglandins are produced by the placenta, the membranes and the fetus, from the action of two enzyme isoforms, the type-1 and type 2 cyclooxigenases (COX-1 and COX-2). COX-2 is constitutive in the fetal

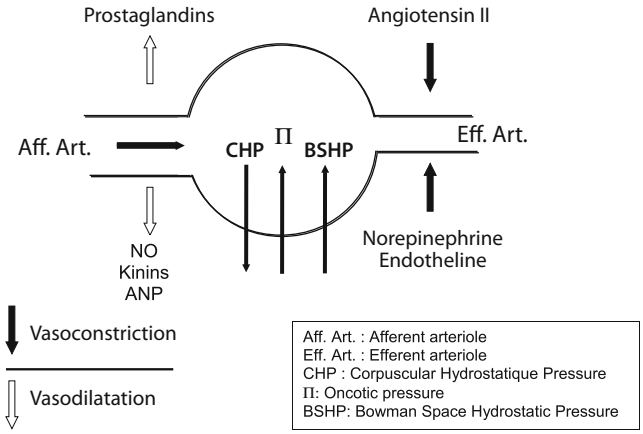

Fig. 124.2 Determinants of glomerular filtration with preglomerular and postglomerular vasoactive forces

kidney and appears essential for the development and function of the kidney [9]. Administration of COX inhibitors during pregnancy decreases renal blood flow, impairs renal function and induces oligohydramnios. Nitric oxide is synthesized by endothelial cells, and vasodilates the afferent arteriole. In the sheep, inhibition of NO leads to increased renal vascular resistance, impaired renal function and reduced sodium excretion. Other factors including endothelin and atrial natriuretic factors can modulate renal hemodynamics.

The expression of vasoactive factors is related to the development of the nephrons. In the rat, as nephrogenesis continues after birth, COX-2, endothelial nitric oxide synthase (eNOS) and angiotensin receptors are highly expressed in immature nephrons and then down-regulated at the end of nephrogenesis. Vasoactive factors participate in the nephrogenesis and in the regulation of intrarenal vascular renal blood flow repartition.

124.2.1.2 Tubular Function

In the adult, the kidney plays an important role in the homeostasis, especially through the function of the tubules. The tubular system is composed of proximal tubules, which are mainly responsible for the regulation of fluid and electrolytes, and of distal tubules and collecting ducts, responsible for fluid and tonicity regulation.

During intrauterine life, the placenta assumes the fetal homeostasis. The maturation of tubular functions follows the development of nephrons. The formation of urine, the main constituent of amniotic fluid in the third trimester, begins by 12 weeks of gestation. The urinary flow rate increases 10 fold from 6 mL/h at 20 weeks to 60 mL/h at 40 weeks of gestation. Fetal urine is hypotonic (100–250 mOsm/kg H_2O). In comparison with maternal plasma, potassium and phosphorus plasmatic levels are higher in fetus suggesting a specific maturation of tubular channels and transporters [8].

The excretion of sodium is higher during fetal life than in the newborn and the adult. Such a high rate of sodium excretion may be related to various factors, including high circulating concentrations and high sensitivity to natriuretic factors; large extracellular fluid volume; relative insensitivity to aldosterone; and dysmaturity of tubular sodium reabsorption. In contrast to the adult physiology, the sodium is mainly reabsorbed in the distal portion of the immature tubules. The sodium/hydrogen exchangers (NHE, four different isoforms) play an important role. These exchangers mediate the exchange of one sodium for one hydrogen ion and contribute to the acidification of urine. The NHE 3 channel, located at the apical or luminal membrane of tubular cells, is thought to be responsible for the bulk of tubular reabsorption of sodium. It is down regulated in the proximal portion of the immature tubules when it is markedly up-regulated after birth. Its expression and activity is dependent on the tubular Na^+, K^+ ATPase, which is down-regulated in the immature kidney [10].

Potassium is important for cell growth and cell function. The balance of potassium is positive in the fetus, due in part to active transplacental transport. Renal excretion of potassium is low and tends to increase towards term. Potassium excretion increases with glomerular and tubular surface area, with maturation of tubular Na^+/K^+ ATPase activity, and with the progressive development of tubular sensitivity to aldosterone.

As for potassium, the fetus is characterized by a positive phosphorus balance resulting from a transplacental transport, a high rate of tubular reabsorption of phosphorus via a sodium-phophorus co-transporter, and a relative parathyroid insufficiency. Calcium is important for the adequate mineralization and growth of the fetal skeleton. Vitamin D-dependent calcium binding proteins, involved in transepithelial calcium transfer, are present in the fetal kidney. The major role of the kidney in fetal calcium homeostasis is the production of $1,25(OH)_2D_3$, rather than the renal regulation of calcium excretion.

During fetal development, the placenta regulates the fetal acid-base balance. The reabsorption of bicarbonates and chloride in the proximal tubule increases with gestational age, which allows the kidney to participate in acid-base homeostasis, near term. This function is mainly related to the maturation of the carbonic anhydrase activity.

The fetus produces hypotonic urine at an elevated rate of 1.5 litres every day. The urine concentration capacity is blunted in the immature kidney. This defect is related to various factors including a low sensitivity of the collecting duct to arginin vasopressin (AVP), a structural immaturity of the loop of Henle with preferential distribution of blood flow to the inner cortex, a low gradient concentration in the medulla due to limited protein intake to generate significant amounts of urea, and a low expression of aquaporin 2 (AQ2). AQ2, a water channel, is located in the apical membrane of collecting duct cells and is involved in water reabsorption. Humans who lack aquaporins have a urine concentrating defect. Expression

of AQs is regulated by the AVP via the V2-receptor. AVP is highly synthesized during the last trimester in pregnancy [11].

Tubular function is dependent on structural maturation of the nephron and on various mediators including the RAS, aldosterone, prostaglandins, atrial natriuretic peptide and cortisol. Cortisol in particular has a potent maturational effect. The sensitivity of renal tubules to such hormonal factors increases along gestation and continues after birth. Such relative tubular immaturity allows the production of amniotic fluid at a sufficient rate and prepares the fetus to the postnatal adaptation [8].

124.2.2 The Newborn

124.2.2.1 Glomerular Function and Renal Blood Flow

GFR is influenced by various factors including blood pressure, renal blood flow and structural determinants. In the neonate, nephrogenesis is completed at birth and the postnatal maturation of glomerular structure consists of an increase in glomerular membrane permeability, in filtration surface area, in corpuscular glomerular diameter (especially in glomeruli from the outer area of cortex), and in intrarenal redistribution of blood flow. Glomerular size reaches adult values at the age of 3 years.

Clearance of endogenous creatinine is widely used to assess GFR in neonates. Creatinine is a metabolite of creatine located in skeletal muscle. Serum levels are correlated with muscle mass. Creatinine is filtered and secreted in part by renal tubular cells. Other markers of GFR including inulin clearance and cystatin C are not used routinely in neonates. Inulin is inert, being not metabolized and neither reabsorbed nor secreted by the renal tubules. Clearance of inulin is the gold standard to evaluate GFR, however it has limited applications in clinical practice. Cystatin C is produced by all nucleated cells and is independent of muscle mass. It is freely filtered, reabsorbed and catabolized in the kidney, which prevents clearance measurement. Serum levels are higher during the neonatal period and stabilize at 12 months of age.

Transition of fetal circulation to extrauterine life is characterized by a rapid increase in systemic blood pressure. In sheep, renal blood flow is low and increases markedly in the first 24 hours after birth rapidly. Renal vascular resistances are elevated immediately after birth and decrease during the first postnatal days. The rapid change in RBF is due to a postnatal decrease in glomerular vascoconstriction, mainly dependent on the RAS. Such hemodynamic changes are associated with increased glomerular capillary hydrostatic pressure, which is still lower than in the adult [12].

At birth, GFR is low compared to adult values, and is correlated with gestational age: 20 mL/min/1.73 m^2 in term and less than 15 mL/min/1.73 m^2 in very low birth weight infants. GFR rapidly increases during the first of month of life (2.5–3 fold increase) (Fig. 124.3) [13]. Postnatal maturation depends on gestational age with a delayed maturation being possibly observed in low birth weight infants (Fig. 124.4). In term neonates plasma creatinine concentration is elevated at birth (60–70 mcmol/L) and stabilizes at the end of the first week (30–40 mcmol/L). In preterms infants, creatinine level at birth is elevated (90–110 mcmol/L), increasing transiently within days 3 to 5 (reaching 130–150 mcmol/L) and then decreases progressively (Fig. 124.5) [14].

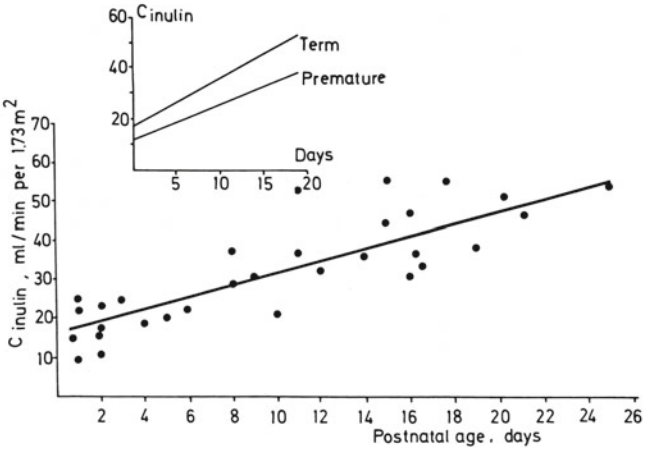

Fig. 124.3 Postnatal glomerular filtration rate in neonates (inulin clearance [C inluin]). Reproduced from [13] with permission

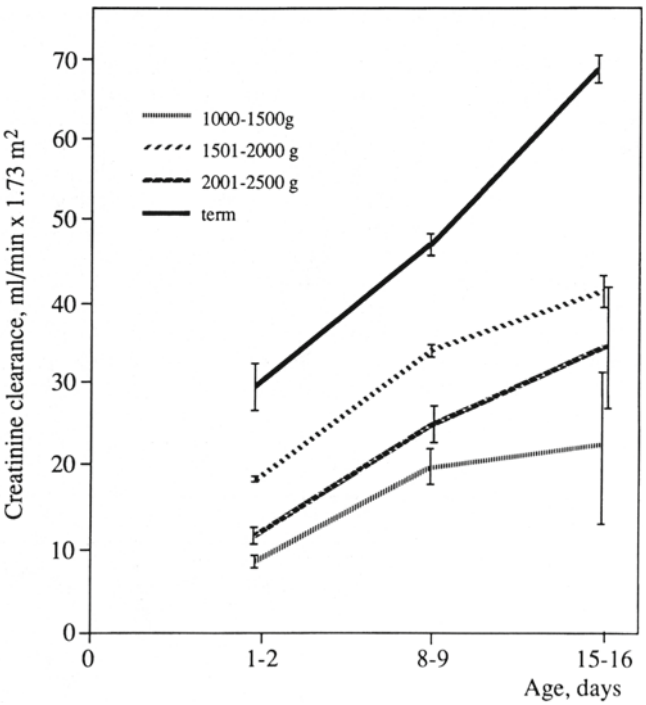

Fig. 124.4 Postantal maturation of glomerular filtration rate in term and preterm infants. Reproduced from [16] with permission

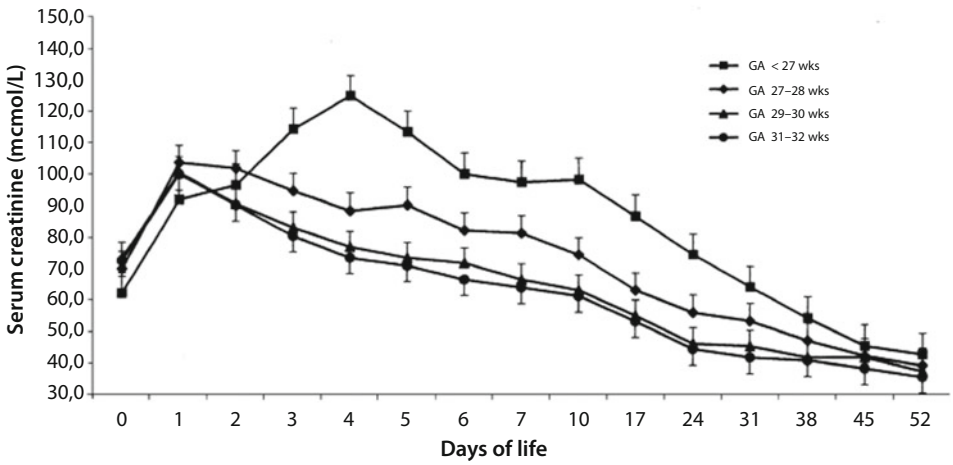

Fig. 124.5 Postnatal maturation of glomerular filtration rate in extremely low gestational age neonates. Reproduced from [14] with permission

GFR is maintained by a delicate balance of vasoconstrictor and vasodilatator forces. Neonates and especially preterm infants are in a state of physiological renal insufficiency. They are at higher risk to develop renal function impairment causes by hypotension, hypovolemia, perinatal asphyxia and nephrotoxic drugs.

124.2.2.2 Tubular Function

The postnatal maturation of renal tubules follows the maturation of GFR. Postnatal maturation is characterized by a 10-fold increase in proximal tubular length and diameter within the weaning period.

After birth, the regulation of homeostasis is transferred from the placenta to the kidney. After birth, postnatal fluid and electrolytic adaptation is characterized by a transient increase in urine output during the first week of life, due to the contraction of the extracellular volume. Commonly, neonates lose around 5–10% of their birth weight. Very low birth weight infants may undergo even a higher weight loss, of up to 15% of birth weight. Fractional excretion of sodium in neonates is around 1%. After birth, maturation of proximal tubules is rapid. In the rat, Na^+/K^+-ATPase activity increases markedly in the first 2 weeks with enhanced expression of proximal NHE exchangers. Fetal and maternal production of endogen glucocorticoids during the neonatal period favours such maturation [8].

The neonate is able to achieve a maximal dilution of urine with urine osmolarity as low as 40–60 mOsm/L. Diluting capacity tends to mature rapidly in the newborn and preterm infants who, at 36th postconceptional age, have a urine dilution capacity comparable to that of adults. Within the limits allowed by GFR, neonates, even preterm infants, tolerate a large range of fluid intake (150 mL/kg/day) without major alterations in water and electrolytes parameters. Diluting capacity is greater than concentrating capacity, which is limited to 400–600 mOsm/L. Children reach the adult maximal urine concentration ability (1300–1400 mOsm/kg) by 2 years of age. This characteristic may be explained by reduced tonicity of the medullary interstitium, low expression of water channels (aquaporins), and a relative tubular insensitivity to ADH. Higher production of prostaglandins E2 in neonates may inhibit the tubular effect of ADH. Low neonatal concentrating capacity is of limited importance in healthy neonates. However neonates and especially preterm infants are more vulnerable to insufficient water intakes and extrarenal water loss (skin loss, diarrhea, etc.), with the risk of osmotic diuresis and hypernatremic dehydration, favored by a lower threshold for renal glucose excretion.

The newborn infant has a diminished threshold for renal bicarbonates excretion. The large expansion of extracellular volume may result in depressed proximal tubular bicarbonate reabsorption. When extracellular fluid is contracted, renal reabsorption increases and urinary pH becomes more acidic. In preterm infants, immaturity of carbonic anhydrase may favour prolonged depressed tubular bicarbonates reabsorption. The normal range for plasma bicarbonates concentration is lower in preterm infants (16–20 mmol/L) than in term infants (21–24 mmol/L).

124.3 Pathophysiology

124.3.1 The Immature Kidney

Nephrogenesis is incomplete in preterm infants. However, recent data have suggested that preterm birth affects postnatal nephrogensis with altered glomerular structure and possibly reduced nephron endowment [15]. Impairment of mesenchymal cell differentiation has been suggested. In comparison with term neonates, GFR is lower in preterm infants and increases progressively after birth [16]. Structural maturation may explain the progressive maturation of GFR in very preterm infants in whom postnatal nephrogenesis continues

after birth. Blood pressure and renal blood flow are low in preterm infants. The immature kidney is particularly dependent on vasodilator forces to maintain a sufficient GFR, allowed by elevated renal vascular resistances from RAS up-regulation. Administration of prostaglandins synthesis inhibitors (indomethacin) for the closure of a patent ductus arteriosus impairs renal function with oliguria, reduced RBF and GFR. Such adverse effect disappears after discontinuation of the drug. Ibuprofen, another COX-inhibitor, is less likely to reduce GFR [17].

Preterm infants are more likely to receive drugs in neonatal intensive care. Most drugs are eliminated by the kidney and undergo glomerular filtration or tubular metabolism, both being immature in preterm infants. Circulating levels of drugs such as vancomycin or aminoglycosids need to be monitor to optimize dosing and reduce toxicity.

Postnatal fluid and electrolytic adaptation is characterized by high water and sodium losses during the first 10 days after birth. Fractional excretion of sodium is elevated in preterm infants (5 *vs* < 1% in term infants) and reach term values progressively after days 15–21. Fluid and electrolytes intakes have to be adapted. Preterm infants are prone to renal water and salt wasting. However, a postnatal contraction of the extracellular fluid volume occurs during the first postnatal days of life and translates into a 2–3% daily, 10–15% total weight loss that should be respected. Moreover the kidney of preterm infants cannot efficiently excrete excessive water and sodium loads, which increases the risk of bronchopulmonary dysplasia, intracerebral hemorrhage and necrotizing enterocolitis. It is thus recommended to introduce sodium, potassium and phosphorus only by postnatal day 2 or 3 and then, when postnatal weight loss is completed, to adapt individually electrolytes intake (especially sodium intake), to maintain a positive balance. Sodium is essential for postnatal growth [18]. Glomerular and tubular consequences of renal immaturity are shown in Table 124.2.

Such glomerular and tubular consequences have been blunted by maternal administration of synthetic glucocorticoids (GC) (betamethasone and dexamethasone). Prenatal administration of GC improves systemic blood pressure, RBF and GFR, and accelerates the maturation of tubular function with enhanced proximal reabsoprtion of sodium and excretion of potassium. Such effects are due to increased expression and activity of sodium exchangers, of Na^+/K^+-ATPase, and to increased renal sympathetic nerve activity [19].

124.3.2 Congenital Renal Tubular Disease

Congenital tubular diseases are rare and include various diseases according to defects in epithelial tubular channels [20]. Bartter syndrome corresponds to a group of tubulopathies involving the regulation and expression of various tubular transporter located in the ascending limb of Henle and the corresponding genes (up-regulation of NKCC2 and ROMK, and down-regulation of Barttin and ClC-Kb). Bartter syndrome is characterized by salt-wasting, by hypovolemia with a low or a preserved arterial blood pressure that activates the RAS, and by metabolic alkalosis. Polyhydramios and hypercalciuria are often observed in fetal and neonatal forms. Treatment consists of adaptation of salt, water and potassium intakes, amiloride and/or indomethacin.

Primary pseudo-hypoaldosteronism is an autosomal recessive disease due to defective epithelial ENaC channel (in collecting ducts). This syndrome is characterized by life threatening salt-wasting, risk of dehydratation and arterial hypotension, severe hyperkaliemia, distal tubular acidosis and hyperaldosteronism. This disease is treated by sodium supplementation. When ENac activity is increased, sodium is exaggeratedly reabsorbed with hypertension, and metabolic hypokaliemia. Renin activity and aldosterone levels are decreased. Liddle syndrome, an autosomal dominant disease, displays such features. Treatment consists in salt restriction and amiloride.

Nephrogenic diabetes insipidus, an X-linked or autosomal transmitted disease, is characterized by polyuria-polydipsia, dehydratation, irritability and failure to thrive. These symptoms are due to a defect in the arginin vasopressin-V2 receptor or in AQ2.

Table 124.2 Glomerular and tubular consequences of immature kidney

Fluid-electrolytes therapy in preterm infants	Glomerular immaturity: consequences	Tubular immaturity: consequences
D1-D2: Volume: 80–100 mL/kg/d Na: 0 K: 0 (except if < 3.5 mmol/L) D3-D5: Volume: 120–140 mL/kg/d Na: 2–4 mmol/kg/d K: 1–2 mmol/kg/d > D5: Volume: 140–170 mL/kg/d Na: 4–6 mmol/kg/d K: 1–3 mmol/kg/d	– Reduced ability to salt/water overload excretion – Hyperkaliemia – High sensitivity to vasoactives drugs – Increased risk of renal failure – Reduced clearance of drugs	– Sodium wasting – Metabolic acidosis – Reduced urine concentration capacity with preserved urine dilution capacity – Glucosuria – Elevated urinary calcium excretion – High sensitivity to diuretics

124.3.3 Parenchymal Consequences of Urinary Tract Anomalies

Congenital abnormalities of the kidney and urinary tract (CAKUT) are a wide range spectrum of anomalies including malformations of uretero-pelvic junction, of vesico-ureteric junction, of vesico-urethral area, and dysplasic kidneys.

124.3.3.1 Renal Structure Changes

The relation between urinary tract obstruction and renal dysplasia is controversial. Renal dysplasia would be the consequence of an induction failure of the metanephrogenic blastema related to urinary obstruction. Obstruction of the collecting system is associated with increase urinary pressure and muscular hypertrophy after an early compliance stage. Early and complete ureteral obstruction in fetal lamb impairs renal architecture with abnormal tubular differentiation, inflammation and reduction of nephron endowment. Changes in renal expression of transcription factors involved in early stage of nephrogensis have been demonstrated in experimental models of fetal ureteral obstruction (Pax2, Wnt). In human, congenital ureteropelvic junction obstruction is associated with renal histologic changes associated with glomerular density, interstitial fibrosis and tubular dilatation [21].

Such complex malformations linking the kidney and urinary tract have been suggested to share common causes. Various defects in specific genes involved in renal development affect kidney parenchyma and urinary tract development (Table 124.1). The quality of the ureteric bud-mesenchyme interaction, the position at which the UB arises from the pronephros, and the pattern of branching morphogenesis are critical for the kidney development. Failure to induce UB outgrowth leads to renal agenesis. An aberrant outgrowth of more than one UB may result in double collecting system and duplication of the ureter. An ectopic positioning of the UB is associated with renal tissue malformation, abnormalities of the uretero-vesical junction, and a defect in the number of branches formed are considered to reduce nephron number endowment at birth (Fig. 124.1).

124.3.3.2 Renal Function Changes

The outcome of CAKUT depends on defects in other organs, on the importance of urinary obstruction and of the alteration of the kidney structure. Early occurrence of urinary tract obstruction, bilateral renal dysplasia, olygohydramnios (with severe fetal renal insufficiency), and reduction of the kidney size across pregnancy are associated with poor prognosis. Biomarkers of renal function in fetal urine have proven of limited interest for the prenatal establishment of prognosis in intermediate cases. Infants with severe abnormalities often die in the perinatal period as a consequence of the Potter's sequence (pulmonary hypoplasia resulting from olygohydramnios and fetal renal function impairement). Survivors may develop renal failure during childhood. Dialysis during the neonatal period, followed by renal transplantation should be discussed with parents and the multidisciplinary nephrology team before and after birth.

Infants with severe obstructive nephropathy are prone to acid-base and hydroelectrolytic balance abnormalities, and to glomerular function impairment. Complete and prolonged fetal uereteral obstruction is associated with decreased RBF and GFR, in proportion to the loss of parenchyma. The RAS is up-regulated in such situations. Infants are prone to type IV renal tubular acidosis (hyperchloremic metabolic acidosis) with hyperkaliemia, salt wasting and defects in urine concentration which are responsible for water wasting and nephrogenic diabetes insipidus. Such tubular dysfunction has been related to changes in tubular channel exchangers characteristics including decreased Na^+/K^+-ATPase activity, unresponsiveness of the damaged distal nephron to aldosterone, with secondary hyperaldosteronism, loss of H^+-ATPase on the surface of intercalated cells, and down-regulation of aquaporins. Treatment of renal infection, adapted protein intake, and limited use or close monitoring of nephrotoxic drugs aim to preserve renal function.

124.3.4 Pharmacological Interactions

The number of pregnant women and women of childbearing age receiving drugs is constant increasing. These drugs can cross the placenta and can impair potentially the function and structure of the fetal kidney. The newborn infant may in turn express renal failure leading to severe renal insufficiency and neonatal death. Selective COX-2 inhibitors and non-selective non steroidal anti-inflammatory drugs (NSAIDs), angiotensin converting enzyme inhibitors ACE-Is and angiotensin type 1 receptor (AT1-R) antagonists are the main drugs affecting the development and the function of perinatal kidney [22].

NSAIDs are used as antalgic or as tocolytic agents (indomethacin) in pregnant women. The renal side effects of in utero exposure to NSAIDs vary from transient fetal oligohydramnios, to severe and lethal renal failure in babies. Quickly after the initiation of indomethacin therapy, a reduction of fetal urine production and oligohydramnios occur. This effect, frequent and transient in many cases, has been the basis of the treatment of polyhydramnios with NSAIDs. The available information originates principally from retrospective and descriptive studies and indicates a rate of incidence from 1.5–20%. Experimental and human studies show that NSAIDs decrease fetal GFR (consequence of renal blood flow reduction) and increase urinary osmolality (enhanced activity of arginin vasopressin). The risk of neonatal renal failure seems increased with prolonged and cumulate doses,

a short time-period between treatment and delivery, pre-existing fetal distress, and low birth weight. In addition to the changes of renal function, a singular renal nephrotoxic dysplasia attributable to chronic antenatal exposure to NSAIDs has been described: the renal mass is reduced, while histologic examination reveals abnormal glomerular and tubular changes including various degrees of ischemic injury and of fibrosis in the medullary area, cortical necrosis, focal tubular and glomerular microcysts of developing nephrons, and loss of differentiation between proximal and distal tubules. Such lesions are associated with increased expression of renin in the juxtaglomerular apparatus. The severity of the renal hypoperfusion induced by NSAIDs may lead to irreversible renal injury involving glomerular and tubular segments, and may distract the development of fetal kidney. Such effects have been noted recently after prenatal exposure to the selective inhibitors of COX-2, a new generation of NSAIDs proposed as an alternative to non specific NSAIDs with the aim of preventing adverse effects.

When administered during pregnancy, inhibitors of the RAS including ACE-Is and AT1-R antagonists, can induce a variety of serious fetal complications, with a high mortality rate. These complications named angiotensin-converting enzyme inhibitor fetopathy include intrauterine growth restriction, profound fetal and neonatal hypotension refractory to any treatment, renal failure, oligohydramnios with limb deformities and pulmonary hypoplasia, hypocalvaria, and increased rate fetal loss. Renal histology shows typically proximal tubular dysgenesia. Numerous cases have been reported with a wide range of adverse fetal and neonatal renal effects varying from transient oligohydramnios (when treatment was arrested rapidly), to severe (requiring peritoneal dialysis) and lethal renal failure. The magnitude of these adverse renal effects cannot be quantified precisely but the risk increases with chronic administration and stage of fetal exposure, especially during the second and third trimester. In contrast, elective exposure during the first trimester of gestation is not associated with an enhanced risk of teratogenicity. When therapy is interrupted rapidly before the 2nd trimester, poor or no adverse renal effects are noted.

Experimental studies have shown that maternal antenatal administration of various drugs can affect nephrogenesis in the absence of clinical renal function impairment in the fetus and newborn infants. In rodents and sheep, antenatal maternal administration glucocorticoids (GCs) at a specific time of pregnancy reduces nephron number and induces hypertension in adult offspring. The mechanism by which GCs alter the fetal kidney structure is unclear. Several pathways are suspected. In utero exposure to GC may 1) disturb the cell differentiation/proliferation ratio at the expense of reduced proliferation, 2) reduce ureteric bud branching, 3) alter gene expression of specific genes involved in kidney development (GNDF), or 4) reduce renal AT1-R and renin gene expression.

Antenatal maternal administration of cyclosporine A (CsA) during gestation (E14–18, and E20–24) affects fetal growth and nephrogenesis leading to a permanent nephron deficit in rat offspring (average of 25–30%). CsA may act through blockage of the conversion of metanephric mesenchyme to epithelium. In humans, outcome of infants exposed in utero to CsA has not shown any changes of renal function and morphology in childhood. But data are too scarce, and long-term follow-up (the maximal age of follow-up was 7 years) is insufficient to conclude definitely in occurrence of side effects in adulthood.

Antenatal maternal administration of ampicillin and aminoglycosides reduces nephron number (20–30%) and induces hypertension in adult rat offspring. Reduced nephron number results from a defect in ureteric bud branching morphogenesis affecting the first branching division. This toxic effect corresponds, in fact, to the first stage of renal development: gentamicin exposure during late stages of nephrogenesis does not induce a nephron deficit. Other aminoglycosides, such amikacin and netilmicin, have less adverse renal effects. Antenatal maternal administration of ampicillin is associated with a 20% reduction of nephron number in rat offspring. An increased rate of apoptosis reaction in mesenchyme area is the suspected leading event of oligonephronia. Such experimental observations have not been found in human.

Other drugs, such as chlorambucil and antineoplasic drugs can impair renal development with urinary tract malformation and renal agenesia.

In most cases, newborn infants do not express clinical renal failure postnatally. However, experimental studies suggest that prenatal exposure to certain drugs may lead to reduced nephron number, followed by hypertension and renal function at adulthood. However, one must be careful in extrapolating data from animals to humans, since major interspecies differences in renal sensitivity to drugs exist, so long-term follow-up of infants exposed in utero to drugs are needed.

124.3.5 Long-Term Consequences of Nephron Number Reduction

It has been suspected for a long time, since the works of Brenner et al in the 80s, that a reduced nephron number is associated with an enhanced risk of hypertension and renal deficiency in adulthood [23]. Reduced nephron number is associated with renal changes including increase in single nephron glomerular filtration rate (SNGFR) and glomerular and tubular hypertrophy. Such hemodynamic changes are responsible for glomerular injury through increase in glomerular capillary hypertension. Over a long time a vicious circle takes place leading to glomerular sclerosis, chronic renal insufficiency and hypertension (Fig. 124.6). Such a hypothesis has been confirmed by experimental studies and human data. Recent data have supported an inverse relationship between reduced nephron number and essential hypertension [24]. An adult born with congenital agenesia is at higher risk of

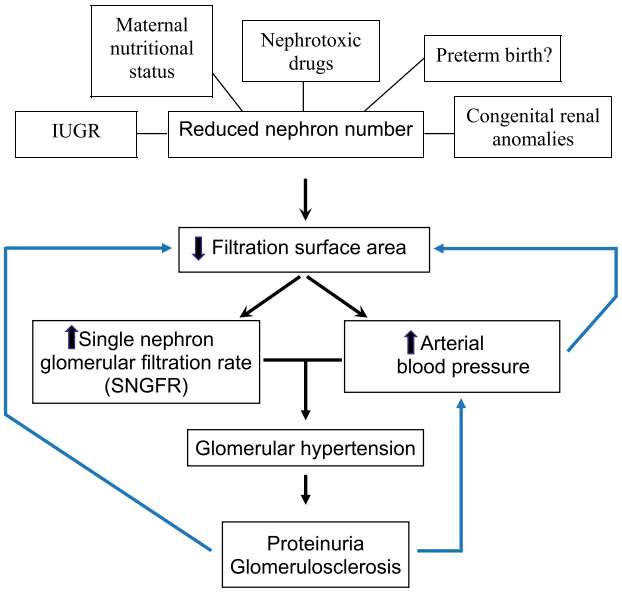

Fig. 124.6 Renal mechanism of systemic hypertension and renal damage, the Brenner hypothesis

developing systemic hypertension and early chronic renal insufficiency [23].

At birth the final nephron number varies from 800,000 to 1 million per kidney. Such variation in nephron number is due to genetic factors and mostly to fetal environment. Various perinatal factors have been shown to induce reduced nephron number including intrauterine growth restriction (IUGR), maternal nutrient deficiency (vitamin A, iron depletion), maternal protein or global nutrition deficit, drugs (see above), maternal and fetal stress, maternal gestational diabetes [2]. In other hand, preterm birth with postnatal adverse environment (postnatal denutrition, stress, nephrotoxic drugs), renal and urinary tract malformations, are associated with impaired nephrogenesis which may lead to reduced nephron endowment.

Such perinatal events are less likely to affect renal functions during the neonatal period but are associated with high risk of hypertension and renal disease at adulthood. Long-term follow-up is needed, with close assessment of blood pressure and renal parameters (creatinine, proteinuria) to prevent adverse evolutive cardiovascular and renal diseases.

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125

Acute and Chronic Renal Failure in the Newborn Infant

Jean-Pierre Guignard and Uma S. Ali

125.1 Introduction

Acute renal failure (ARF) is the consequence of a sudden decrease in glomerular filtration rate with consequent retention of nitrogen waste products and disturbances in water, electrolyte and acid-base balance. Because of its physiological specifics, the premature fetal and neonatal kidneys are particularly vulnerable to common endogenous and exogenous stresses occuring before birth, during delivery or after birth. Clinical studies have shown that 8–24% of all neonates hospitalized in intensive neonatal care units may present with ARF [1–4]. The causes of ARF (Table 125.1) are divided into prerenal (functional), renal (intrinsic) and postrenal (obstructive) causes. Functional pre-renal insufficiency is the most frequent form of ARF. It is preventable if treated early and adequately.

125.1.1 The Fetal Kidney

The fetal kidney lives in a protected environment without real homeostatic responsibilities. Perfusion is limited, the fetal kidney receiving only 3–4% of the cardiac output. Glomerular filtration rate (GFR) is also extremely low in the fetus. Nephrogenesis is complete by 35 weeks of gestation. At birth, the immature kidney is abruptly thrust into an independent extrauterine existence where it has to assume the responsibility of safeguarding the life and well-being of the neonate by providing the moment to moment fine tuning required for body homeostasis [5].

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Table 125.1 Normal mean arterial blood pressure in the newborn infant*

Age	< 1.0 kg	1.0–1.5 kg	> 2.5 kg
Birth	33 ± 15	39 ± 18	49 ± 19
1 week	41 ± 15	47 ± 18	60 ± 19
2 weeks	45 ± 15	50 ± 18	64 ± 19
4 weeks	48 ± 15	53 ± 18	68 ± 19

* Mean ± 95% confidence limits for single measurements; measurement via umbilical artery catheterization or by Dynamap. Adapted from [22].

125.1.2 The Neonatal Kidney

Glomerular filtration rate at birth is close to 20 mL/min per 1.73 m^2 in term neonates [5]. It doubles within 2 weeks and reaches 50 mL/min per 1.73 m^2 at 4 weeks of life (Fig. 125.1) [5, 6]. The neonatal kidney is thus in a state of relative physiological renal insufficiency during the first postnatal month and is ill-equipped to handle major homeostatic challenges. The driving force for producing the glomerular filtrate is the systemic arterial pressure which is quite low in the newborn period (Table 125.1).

The two major vasoactive forces that optimize the driving force for filtration are the prostaglandins that relax the afferent arteriolar tone and angiotensin II (ATII) that constricts the efferent arteriole, thus increasing the effective transglomerular pressure gradient (Fig. 125.2). This hydrostatic pressure is opposed by the intratubular pressure as well as by the oncotic pressure, resulting in a net driving force of only a few mmHg [2]. GFR shows a progressive direct correlation with both gestational and postnatal ages [5, 6].

Premature birth increases the renal vulnerability. In healthy premature neonates, nephrogenesis continues after birth, but for only 40 days [7]. Nephrogenesis stops at birth in growth-retarded neonates. Low birthweight infants have consequently a lower number of nephrons as compared to term infants. GFR is lower in low birthweight infants and increases more slowly than in full-term neonates.

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

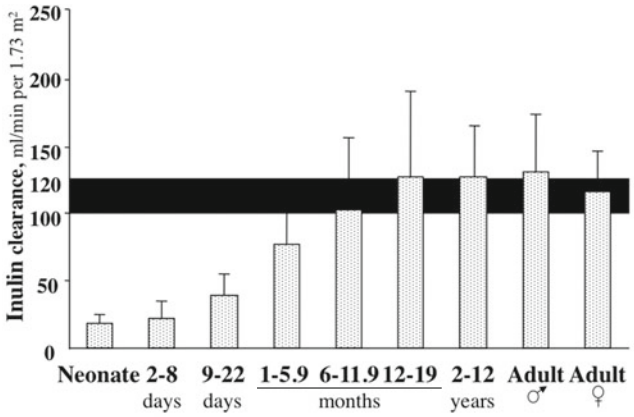

Fig. 125.1 Development of glomerular filtration rate (GFR)

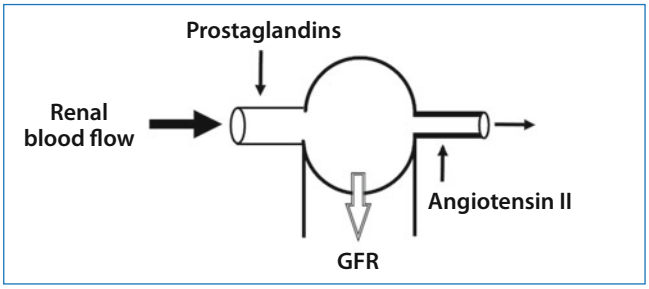

Fig. 125.2 The glomerulus: intrarenal regulation of GFR

125.1.3 Definition

Acute renal failure can be oliguric or non-oliguric, so that urine flow rate cannot define ARF, the diagnosis of which is based on the follow-up of plasma creatinine concentration. ARF is suspected when the plasma creatinine is above 130 µmol/L (15 mg/L) for at least 24–48 h while maternal renal function is normal. In very premature neonates, the elevated plasma creatinine present at birth increases in the first 3–4 days of life because of tubular reabsorption of creatinine across leaky tubules [8]. In clinical practice, repeated measurements of plasma creatinine are used to confirm the presence of ARF.

Oliguria is defined by an urine output less than 1.0 mL/kg per h in preterm and less than 0.5 mL/kg per h in term neonates.

125.2 Etiology and Pathogenesis

125.2.1 Etiology

Risk factors for the development of ARF include perinatal asphyxia, antepartum hemorrhage, sepsis, hyaline membrane disease, administration of non-steroidal anti-inflammatory drugs (NSAIDs) pre- or post-natally and hypernatremic dehydration (Table 125.2) [9]. The primary disease may manifest clinically as respiratory distress in hyaline membrane disease or as lethargy, apnea, poor feeding, prolonged capillary refill and hypotension in sepsis. More than 75% of cases of neonatal renal failure are prerenal in nature. Intrinsic renal failure accounts for less than 10% of the cases and may occur due to drug-induced nephrotoxicity or when the hypoxic-ischemic insults are prolonged or severe [4].

125.2.1.1 Prerenal Causes

All factors that decrease cardiac output can lead to ARF. Some insults to the kidney begin in prenatal life. Fetal hypovolemia as a result of feto-fetal transfusions, feto-maternal transfusions and ante-partum hemorrhage can lead to renal ischemia in utero with or without fetal hypotension resulting in fetal oliguria, oligohydramnios and postnatal renal failure. Hypotension, hypoxemia, metabolic acidosis, metabolic alkalosis, small changes in body temperature, as well as the administration of vasoconstrictive agents are common stresses that increase renal vascular resistance [2]. Cardiac diseases, the persistence of patent ductus arteriosus and hyperviscosity states can also impair cardiac output [1].

Table 125.2 Causes of acute renal failure in neonates

Prerenal
- Hypovolemia or renal hypoperfusion
- Asphyxia
- Respiratory distress syndrome (RDS)
- Dehydration
- Hemorrhage (maternal antepartum, twin-to-twin transfusion, intraventricular bleeding, hemolytic disease)
- Sepsis
- Cardiac disease (patent ductus arteriosus, aortic coarctation)
- Polycythemia (hyperviscosity)

Renal
- Acute tubular necrosis (ATN)
- Persistent prerenal disturbances
- Nephrotoxins (nephrotoxic antibiotics, e.g., aminoglycosides, contrast agents, angiotensin-converting enzyme [ACE] inhibitors and angiotensin II AT1 receptor blockers)
- Myoglobinuria, hemoglobinuria, hyperuricemia
- Vascular disorders (renal vein thrombosis, renal artery thrombosis, aortic thrombosis, disseminated intravascular coagulation)
- Congenital renal anomaly (dysplasia, hypoplasia, polycystic kidney, agenesis)
- Pyelonephritis
- Transient acute renal failure of the neonate
- Maternal etiology (gentamicin, indomethacin, ACE inhibitors, paraproteinemia)

Postrenal
- Congenital anomaly (ureteral or urethral obstruction, neurogenic bladder, magacystis-megaureter)
- Obstruction secondary to circumcision
- Renal candidiasis
- Calculi
- Neurogenic bladder

125.2.1.2 Intrinsic Causes

Persistent prerenal disturbances can lead to acute renal necrosis and severely impaired renal function [3, 4]. The renal parenchyma can also be injured by vascular disorders, renal thrombosis, nephrotoxins, acute pyelonephritis, myoglobinuria and hemoglobinuria. Cortical necrosis may result from severe ischemia and microthrombi formation. Congenital renal anomalies, including hypodysplasia and cystic diseases can favor the occurrence of both ARF and chronic renal failure.

125.2.1.3 Postrenal Causes

Obstruction on the urinary tract, whatever the cause, can lead to ARF. The obstruction can occur at the level of the ureters, the bladder or the urethra. When not relieved immediately, the obstruction can induce permanent damage to the kidney [10].

125.2.1.4 Nephrotoxic Drugs

Nephrotoxicity of drugs may play an important role in producing renal injury [11]. They can affect the fetus when administered to the pregnant mother, or the neonate when administered post-natally. Aminoglycosides may cause renal injury even with normal blood levels due to the high tissue concentrations of the drugs that can be achieved. All cephalosporins are potentially nephrotoxic though clinically the third generation cephalosporins appear to be reasonably safe. Vancomycin needs to be used in appropriate dosages and monitored closely. The use of two simultaneous nephrotoxic drugs should preferably be avoided. Vasoactive drugs interfering with the synthesis of prostaglandins as well as with the renin-angiotensin system are particularly deleterious to the immature kidney [2, 4].

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

The maintenance of prenatal as well as postnatal GFR is highly dependent upon prostaglandin-mediated afferent arteriolar dilatation [2]. Inhibition of prostaglandins can decrease renal perfusion and GFR [12], and even lead to severe fetal or neonatal failure that can even be irreversible [13]. In utero exposure to the non-selective NSAIDs inhibitors such as aspirin, indomethacin and ibuprofen have indeed been associated with adverse fetal outcomes such as fetal olguria and neonatal renal failure. Administration of NSAIDs early in pregnancy carries a high-risk of inducing renal tubular dysgenesis in the fetus, with fetal oliguria and oligohydramnios. COX-2 is indeed also expressed in the proximal tubular cells and is believed to play an important role in the differentiation of the renal tubules.

The NSAIDs are cyclo-oxygenase (COX) inhibitors, some of which non-selectively inhibit COX-1 and COX-2, while others selectively inhibit COX-2. Because COX-1 is constitutively expressed in several cell types in the gastrointestinal tract, liver, platelets and kidney, toxicities are fairly commonly encountered when it is inhibited. COX-2 is an inducible enzyme and is not expressed in the gastrointestinal tract and liver. COX-2 inhibitors were thus expected to have a better safety profile. Experimental and clinical studies on nimesulide, a COX-2 selective inhibitor, have shown that the drug can also produce renal tubular dysgenesis and failure when administered pre- or postnatally [14]. When really needed, all NSAIDs must be used with great caution, be it before or after birth [2].

Angiotensin Inhibitors Converting Enzyme Inhibitors (ACEIs) and Angiotensin II AT1 Receptor Blockers (ARBs)

The renin-angiotensin system is highly activated in fetal and neonatal life and the maintenance of a normal GFR in the face of physiologically low arterial pressures is dependent on efferent arteriolar vasoconstriction mediated by angiotensin II [2]. Interfering with the action of angiotensin II by maternal ingestion of ACEIs or ARBs can result in a dramatic fall in GFR leading to fetal oliguria, oligohydramnios and postnatal oliguric renal failure that can be irreversible. Angiotensin II receptors type 2 are expressed prominently during fetal life in the proximal tubules and play an important role in tubular differentiation. Maternal ingestion of ACEIs and ARBs produces a classical fetopathy with renal tubular dysgenesis [15]. Postnatal administration of drugs interfering with the action of angiotensin II can also depress GFR and lead to renal failure. Drugs interfering with angiotensin II must definitively be avoided during pregnancy and used with caution after birth.

Miscellaneous Drugs and Iatrogenic Intervention

Tolazoline, an alpha-adrenergic blocking agent used in the past as a pulmonary vasodilator, as well as D-tubocurarine or pancuronium can increase renal vascular resistance [2].

Ventilation with continuous positive airway-pressure has deleterious effects on renal function due to decreased venous return and low cardiac output, increased renal sympathetic nervous activity and very elevated serum vasopressin levels [2].

125.2.2 Specific Pathogenesis

The hemodynamic effects of stresses such as preterm birth, asphyxia, respiratory distress and sepsis lead to decreased systemic arterial pressure, diminished renal blood flow and a marked increase in the renovascular resistance (Fig. 125.3). These changes further reduce the already small net effective filtration pressure, leading to a clinically deleterious fall in GFR [2].

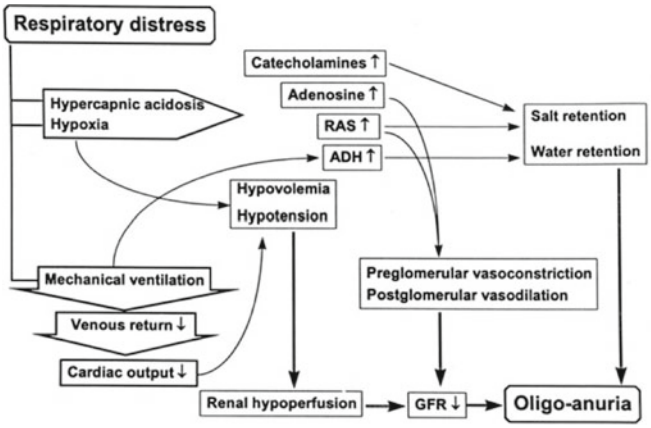

Fig. 125.3 Pathogenesis of hypoxic renal failure. Mechanical ventilation as well as the hypoxemia-induced activation of vasoactive factors contribute to the development of renal hypoperfusion, renal insufficiency, and oligoanuria. Reproduced from [2] with permission

125.2.2.1 Hypoxemic-Hypoxic Insults

Hypoxia is one of the commonest stresses faced by the neonate and it leads to the activation of various potent intrarenal vasoconstrictors such as the catecholamines, angiotensin II, adenosine and endothelin, which play a role in the pathogenesis of ARF. Hypoxic-ischemic insults also activate the intracellular hydrolysis of ATP that generates adenosine. Studies in newborn rabbits have shown that adenosine causes constriction of the afferent arterioles and relaxation of the efferent arterioles leading to a profound drop in GFR. Pretreatment with theophylline, a non-specific adenosine antagonist, prevents the development of ARF in hypoxic animals and asphyxic neonates [16]. Endothelin is a potent vasoconstrictor but its role in the pathogenesis of vasomotor nephropathy is still inconclusive [13]. The occurrence of renal failure in severely asphyxiated neonates is extremely frequent, in up to two-thirds of patients [1–4].

125.2.2.2 Sepsis

ARF in sepsis is part of multiorgan dysfunction and is mediated by both hemodynamic and non-hemodynamic factors. Sepsis-mediated cardiorespiratory depression results in renal hypoperfusion and a decline in GFR that is sustained by various vasoactive substances such as angiotensin II, the catecholamines, adenosine, endothelin and thromboxane A2 [17]. Renal failure can occur in septic states in the absence of altered global hemodynamics. Bacterial lipopolysaccharides (LPS) along with proinflammatory cytokines, free-oxygen radicals, procoagulant and immunological factors generated by the host both intra- and extrarenally contribute to the development of ARF.

Platelet activating factor (PAF) produced by LPS-stimulated resident renal cells as well as by polymorphonuclear leucocytes, plays an important role in the pathogenesis of ARF, producing a fall in renal blood flow and GFR when infused in animals. Intrarenal production of endothelin-1 (ET-1) stimulated by tumor necrosis factor produces a marked fall in renal blood flow and GFR that is ameliorated by administration of ET-1 antibodies or receptor blockers. Endotoxemia also increases the renal expression of inducible nitric oxide synthase mRNA that leads to a fall in blood pressure and decrease in GFR [18, 19]. The intermediate host response to the inciting agent thus results in the sequential production of several inflammatory cytokines and chemical mediators that facilitate the progression of the inflammatory cascade resulting in multiorgan dysfunction and renal failure.

125.3 Clinical Events

Clues to the presence of renal dysfunction may manifest in prenatal life as oligohydramnios. As fetal urine is the major contributor to the amniotic fluid volume, the occurrence of olighydramnios may suggest fetal oliguria and the presence of severely affected kidneys in utero. Bilateral renal agenesis, multicystic dysplastic kidneys, autosomal recessive polycystic kidneys (ARPKD) and obstructive uropathies can all lead to olighydramnios. Late onset olighydramnios is a characteristic finding in renal tubular dysgenesis and warrants a thorough evaluation for exposure to drugs such as NSAIDs, ACEIs and ARBs. The technological and pharmacological interventions used to improve the outcome of sick neonates may themselves contribute to the occurrence of ARF.

Mechanical ventilation reduces the preload by increasing the intrathoracic pressure and results in decreased cardiac output and renal perfusion. Mechanical ventilation *per se* may generate cytokines and chemical mediators that lead to multiorgan dysfunction [20]. Vasoactive infusions used to maintain normotension may lead to excessive splanchnic vasoconstriction, renal ischemia and ARF. Umbilical artery and vein catheterization can lead to renal artery or renal vein thrombosis. Indwelling urinary catheters favor the occurrence of urinary tract infections and the use of broad-spectrum antibiotics may result in the development of fungal urinary tract infections (UTIs) and fungal bezoars [1].

125.3.1 Physical Features

The presence of external features such as Potter's facies, preauricular tags, polydactyly, single umbilical artery or sacral abnormalities may indicate the presence of an underlying developmental renal anomaly. Enlarged kidneys may indicate autosomal polycystic renal disease or obstructive uropathy. Palpable bladder and a poor stream of urine in a male child

suggest the presence of posterior urethral valves. Although these congenital uropathies may present as acute renal failure they are chronic problems that tend to be persistent or progressive in nature [21].

Edema due to fluid overload, hypoproteinemia or capillary leak may complicate the clinical course. Hypotension due to hypovolemia, sepsis or cardiac dysfunction may coexist. Hypertension due to renal failure may be encountered during the course of illness as a result of volume overload or a high renin state. Recognition of hypertension requires reference to appropriate charts as the normal range of blood pressure in neonates would vary depending on gestational age, weight and postnatal age (Table 125.1) [22].

125.3.2 Oliguric ARF

Thirty percent of normal newborns void soon after birth; 92% will void within the first 24 h and 99% by 48 h. Oliguria is observed in the majority of neonates presenting with ARF. Oliguria can also result from poor fluid intake or be due to inappropriate ADH secretion in sick neonates.

125.3.3 Non-Oliguric ARF

One fourth to one third of neonates with ARF may have non-oliguric failure and the renal failure can only be recognized by the finding of elevated urea and serum creatinine concentrations. Birth asphyxia, aminoglycoside therapy and methoxyflurane anesthesia are some of the conditions leading to non-oliguric renal failure [23].

125.4 Laboratory Evaluation

125.4.1 Urinalysis

Urinalysis in vasomotor nephropathy may show normal urine, epithelial cells and coarse granular casts, normal urine, or mild proteinuria, microscopic hematuria, epithelial cells and coarse granular casts. Gross or microscopic hematuria is usually seen in renal vein thrombosis and sometimes in urinary tract infections. Pus cells or pus cell casts reflect severe UTI.

125.4.2 Blood Count

Anemia is present in neonates with ARF, in whom it may also reflect bleeding or dilution by fluid retention. Eosinophilia suggests an allergic reaction to drugs.

125.4.3 Plasma Urea

Azotemia refers to the retention of nitrogenous waste products and is detected by the finding of elevated levels of plasma urea and creatinine. Plasma urea is a poor marker of renal function as it is influenced by multiple factors such as protein intake, urine output, gastrointestinal bleeding and a hypercatabolic state. It may be deceptively low in sick neonates as a result of poor protein intake.

125.4.4 Plasma Creatinine

Creatinine is the most widely used surrogate marker for GFR albeit not a perfect one as it is not only filtered by the glomeruli but also secreted by the renal tubules in term neonates. The serum creatinine in the first few days of life reflects maternal creatinine levels and gradually comes down to normal neonatal levels close to 4 mg/L (35 μmol/L) by one week of age in term infants. In preterm babies the creatinine may actually rise further before falling slowly to normal neonatal levels over 2–3 weeks (Fig. 125.4). [24, 25]. This increase occurs because of back-leak of filtered creatinine through leaky immature tubules [8]. Failure of plasma creatinine to decrease below maternal levels after the first few days of life or a rise in serum creatinine greater than 25–30 μmol/L (3 mg/L) per day is indicative of acute renal failure [1].

Creatinine levels may also be artefactually elevated depending on the associated co-morbid conditions as well as the methodology employed for its estimation. In the commonly used Jaffe's method that also measures non-creatinine chromogens, creatinine levels may be spuriously high in the presence of hyperbilirubinemia or treatment with cephalosporins. However, the use of newer techniques such as the kinetic or

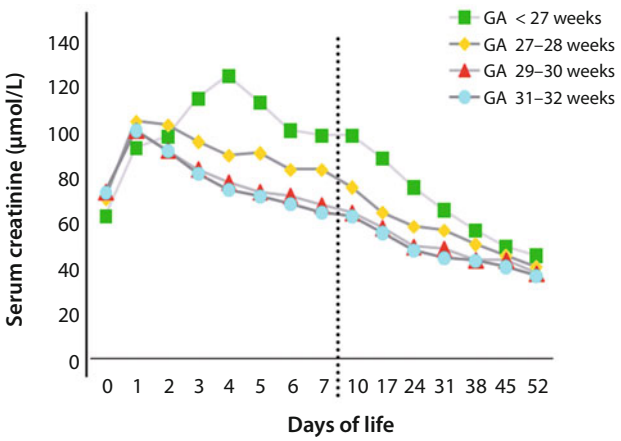

Fig. 125.4 Plasma creatinine in the first weeks of life of premature neonates. Reproduced from [24] with permission

enzymatic assays does not measure these chromogens and give truer values of creatinine. Further improvement in measuring creatinine has recently been achieved by the use of newer techniques such as the high-performance liquid chromatography and the gas chromatography-isotope dilution mass spectrometry [26].

125.4.5 Plasma Cystatin C

Cystatin C, a non-glycosated 13-kDa basic protein produced by all nucleated cells has been claimed to be a better glomerular marker than creatinine. Cystatin C is freely filtered, almost completely reabsorbed and catabolized by proximal tubular cells. However, the clinical use of this marker suffers important drawbacks:

a. the handling of this product by the immature tubular cells, in particular when they are injured is not known;
b. its production and concentration is influenced by factors independent of GFR, such as rises in serum protein C-reactive levels, thyroid dysfunction or corticosteroid administration; and
c. its measurement is considerably more expensive than that of creatinine, by a factor of at least 12 [26].

125.4.6 Urinary Indices

Chemical analysis of urine may help in distinguishing prerenal from intrinsic ARF. Previously healthy kidneys respond to acute hypoperfusion by maximally conserving water and electrolyte, thus excreting concentrated urine with low sodium concentration (U_{Na}). By contrast an intrinsically damaged kidney is not able to concentrate urine and retain sodium. Therefore a urinary osmolality (U_{osm}) above 400 mosm per kg H_20, a U_{Na} below 40 mmol/L and a fractional excretion of sodium (FE_{Na}) below 2% suggest the presence of a prerenal functional problem. The assessment of FE_{Na} is more reliable than the U_{Na} only. It should be noted that these urinary indices are only valid when measured before the administration of volume expanders or diuretics, and that they cannot be used in very premature salt-wasting neonates who may normally present with FE_{Na} above 5% [1–4].

125.5 Imaging

Imaging of the genitourinary tract by ultrasound is useful for assessing the size, the shape and the echogenicity of the kidneys; for diagnosing pelvicalyceal dilatation; and for examining the ureters and the bladder. Imaging is almost an extension of the clinical examination and gives highly valuable diagnostic information. Evaluation of the renal vessels by color Doppler gives important information regarding the presence of renal vein or renal arterial thrombosis. Radionuclear scan may be useful in suspected cases of obstructive uropathy.

125.6 Conservative Management

125.6.1 Management of Suspected Prerenal Oliguric ARF

Acute renal failure should be proactively detected in the neonatal intensive care unit by regular monitoring of urine output and serial measurements of serum creatinine. As the cause of ARF is generally multifactorial, reassessment of both the primary problem and all the therapeutic interventions needs to be done in order to identify the contributory factors. When feasible, nephrotoxic drugs should be substituted by non-nephrotoxic agents. Drug dosages should to be adjusted to the presupposed level of GFR [27] (see Table 125.3).

125.6.1.1 Fluid Challenge and Rehydration

In the prerenal types of ARF, restoration of volume by the administration of 20 mL/kg of normal saline over 1–2 h usually improves urine output (see Fig. 125.4). Such administration should not be given when there is clinical evidence of fluid overload or strong suspicion of a cardiac cause for the prerenal failure. In neonates with fluid refractory hypotension, dopamine infusion should be started to improve blood pressure and renal perfusion. Low-dose hydrocortisone may be useful in fluid and dopamine refractory hypotension [28].

128.6.1.2 Low-Dose Dopamine

The use of low, non-pressor doses of dopamine in preterm babies with hyaline membrane disease has been claimed to im-

Table 125.3 Drugs requiring dose adjustment when GFR is compromized

Antibiotics
– Aminoglycoides (amikacin, gentamicin, totramycin)
– Amoxycillin, amoxycillin-clavulanic acid
– Cephalosporin (cefixime, cefotaxime, ceftazidime)
– Cotrimoxazole
– Ticarcillin
– Vancomycin
Antifungial agents
– Fluconazole
Cardiac drugs
– Digoxin

prove renal blood flow, natriuresis and diuresis, and produce a modest improvement in renal function [29]. The renal vasodilatory and diuretic response presumably results from stimulation of dopaminergic receptors that occurs with low-dose dopamine. Higher doses lead to alpha-receptor stimulation that causes systemic vasoconstriction as well as an increase in renal vascular resistance. What constitutes a renal vasodilator dose in a given patient is not always clear and has varied from 2.5–6 μg /kg per min. Variations in the dopaminergic receptor density, increase in half-life of dopamine, decreased adrenoreceptor sensitivity mediated by extreme prematurity, systemic hypoperfusion and other pathological states may be responsible for the variations in the renal vasodilatory dose. What may be a vasodilator dose in one baby may be a pressor dose in another. Dopamine can have several adverse effects such as tachycardia, cardiac arrhythmia and increase in pulmonary vascular resistance. The modest benefits that may be obtained in some babies with low-dose dopamine have to be weighed against the possible adverse effects of the drug. Noteworthy is the fact that all neonatal studies in favor of the renal doses of dopamine were uncontrolled, and that a placebo-controlled randomized study in adult patients failed to demonstrate that low-dose dopamine could protect the stressed kidney [30].

125.6.1.3 Furosemide

When urine output fails to improve after fluid repletion, a trial of diuretics can be given. Intravenous furosemide at 1–3 mg/kg may improve the diuresis. The natriuretic and diuretic responses to furosemide are highly variable and depend on the level of GFR. When a diuretic response is present, it carries the risk of inducing hyponatremia, with consequent vasoconstriction of the kidney. Torasemide, a loop diuretic with close similarities to furosemide, is sometimes used instead of furosemide. When given to immature rabbits undergoing hypoxemic vasomotor nephropathy, torasemide has been shown to increase sodium excretion and urine output without improving renal perfusion and GFR [31]. Clearly, loop diuretics should basically not be used to treat oliguric neonates, but primarily those presenting with edematous states and congestive heart failure. When given together, dopamine and furosemide have been claimed to have a synergetic effect in increasing urine output. It remains questionable whether this would also improve renal function.

125.6.1.4 Mannitol

Mannitol is best avoided in the neonate as it can increase intravascular volume and produce fluid overload in the absence of diuresis. Being hyperosmolar it should be especially avoided in premature babies as it may give rise to intracranial hemorrhage. Mannitol itself can cause or exacerbate ARF.

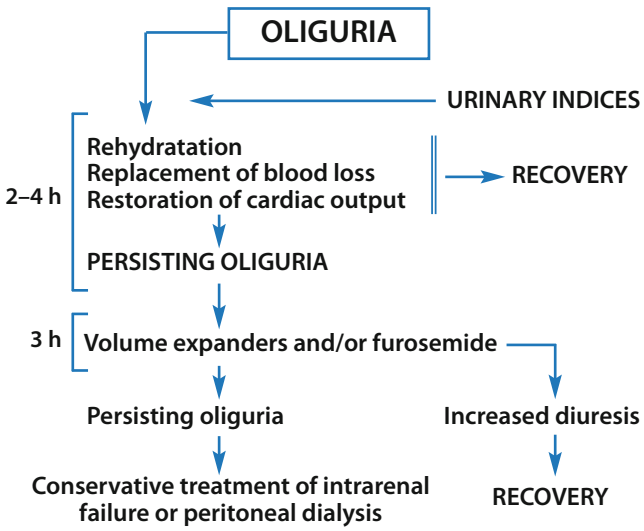

Fig. 125.5 Management of acute renal failure

125.6.2 Fluid Management in Neonates with Intrinsic ARF

Oliguric neonates who fail to respond to the above maneuvers (Fig. 125.5) should be considered to have intrinsic renal failure and require additional measures to prevent major homeostatic disturbances [32]. In those neonates responding to the above measures by an increase in urine output, a progressive fall in the serum creatinine is observed. Persistence of elevated creatinine, or increases in creatinine levels in the face of a good urine output suggests the presence of non-oliguric renal failure. In a normal neonate with intact renal function urine output contributes to 50–80% of the total fluids administered and varies with the amount of solute needed to be excreted. In oliguric neonates, fluid administration should be restricted to insensible water losses plus the ongoing renal and extrarenal losses. The insensible water losses in full-term neonates amounts to 35 mL/kg per day whereas in preterms this may vary from 60–70 mL/kg per day. The fluid administered should be electrolyte-free and the concentration of glucose adjusted to deliver the adequate amount of glucose. Daily weight is the best parameter to judge adequacy of fluid management. In oligo-anuric neonates a daily weight loss of 0.5% is expected and reflects appropriate fluid balance.

125.6.3 Electrolyte Disorders

125.6.3.1 Hyponatremia

Hyponatremia when present is dilutional in nature and should not be treated by giving sodium chloride. Free-water admin-

istration should be restricted instead to produce a gradual increase in the serum sodium concentration. However, when the serum sodium level is less than 120 mmol/L, it can give rise to cerebral edema that may manifest as lethargy, seizures and apnea. In such situations 3% NaCl may be given iv at a slow rate of 5–6 mL/kg over 2–4 hours. The amount of sodium required to correct the hyponatremia can also be estimated by the formula:

$$\text{Na (mmol)} = [\text{desired Na-actual Na (mmol/L)}] \times (0.8 \times \text{BW})$$

where BW represents body weight (in kg) and 0.8 × BW represents total body water.

125.6.3.2 Hyperkalemia

Elevated potassium levels are fairly common in ARF. Care should be taken to confirm that potassium has been eliminated from the iv infusions and that medications containing potassium are not administered. Mild to moderate hyperkalemia of 6–6.5 mmol/L without electrocardiographic (ECG) changes may require nothing more than the correction of co-existing metabolic acidosis. Higher levels of potassium or the presence of ECG changes require active cardio-protective measures that include intravenous calcium administration (0.5–1.0 mL/kg of 10% calcium gluconate, over 5 min under ECG surveillance), and glucose-insulin infusions. The benefit of adding inhaled albuterol to the infusion of glucose-insulin awaits confirmation. When the neonate is still passing urine, diuretics such as furosemide (1 mg/kg) can be tried. The use of potassium binding resins is not without risk in infants. It may cause gastric erosions when given orally and colonic ulcerations and impaction when given rectally. They are best avoided in a sick neonate [33]. Severe hyperkalemia (> 7.5 mmol/L) and/or major ECG changes represent an indication for dialysis.

125.6.3.3 Hypocalcemia

Hypocalcemia is common but the ionic fraction may not be very low because of concomitant hypoalbuminemia and metabolic acidosis. When the hypocalcemia is symptomatic intravenous calcium gluconate (10% calcium gluconate at a dose of 0.5–1.0 mL/kg in 5 min under cardiac monitoring) needs to be given and repeated every 6–8 h. Caution is required in the administration of iv calcium in the presence of hyperphosphatemia, as it may lead to soft tissue calcification and nephrocalcinosis.

125.6.3.4 Hyperphosphatemia

Hyperphosphatemia is common in neonates with renal failure. Calcium-containing phosphate binders such as calcium carbonate may be used although severe hyperphosphatemia is best treated with dialysis. Aluminum containing phosphate binders are best avoided.

125.6.3.5 Acidosis

Mild metabolic acidosis is common in renal failure. When the plasma bicarbonate concentration falls below 12 mmol/L and/or the plasma pH below 7.20, correction of the acidosis is best achieved by adding sodium bicarbonate to the maintenance fluid.

125.6.4 Convulsions

Convulsions are the consequence of electrolyte imbalance or arterial hypertension. Symptomatic therapy is achieved by giving diazepam (0.2 mg/kg iv or rectally), iv lorazepam (0.05–0,1 mg/kg), or iv phenobarbital (15–20 mg/kg).

125.6.5 Hypertension

Hypertension may occur as a result of volume overload as well as hyperreninemia.

Severe or symptomatic hypertension is best treated with continuous iv infusion of nicardipine. Esmolol, enalaprilat, or sodium nitroprusside can be used with caution (Table 125.4) [1, 4]. The optimal oral therapy for newborns is not clearly known. ACE inhibitors notably captopril has been used in neonatal hypertension. However its use in the context of neonatal renal failure is risky. If necessary it should be used cautiously in small doses. Calcium channel blockers can be used as first line drugs. Beta blockers should be used with caution and are best avoided in babies with coexisting respiratory problems. In these babies diuretics may form a good adjunct.

125.6.6 Sepsis

Severe infection is a common cause of death in neonates with ARF. Adequate intravenous antibiotherapy is essential. The dosage of drugs excreted mainly by the kidney (aminoglycosides, vancomycin) must be adapted to the severity of renal failure. When possible, monitoring of plasma concentrations is recommended.

Table 125.4 Antihypertensive drugs in the neonate

Agent	Starting dosage	Intervals	Maximum recommended	Route of administration
Furosemide	1 mg/kg	q 4–6 h	5 mg/kg/day	O/IV
Hydrochlorothiazide	1 mg/kg	q 8 h	3 mg/kg/day	O
Propranolol	0.25 mg/kg/dose	q 6-8 h	5 mg/kg/day	O/IV
Atenolol	0.5 mg/kg/dose	q 12–24 h	4 mg/kg/day	O
Labetalol	0.5 mg/kg/dose	q 1–4 h	2 mg/kg/day	IV
Sodium nitroprusside	0.5 µg/kg/min	–	6 µg/kg/min	IV
Captopril	0.1 mg/kg/dose	q 8–12h	0.5 mg/kg/day	O
Enalapril	5 µg/kg/dose	q 8–24 h	20 µg/kg/day	IV
Nifedipine	0.5 mg/kg/dose	q 4–6 h	2 mg/kg/day	O

125.6.7 Nutrition

Unless aggressively managed, neonatal renal failure invariably results in nutritional failure. Nutritional inadequacy is an important contributor to neonatal mortality. Enteral nutrition when feasible should consist of appropriate formulae that are calorically dense with a low electrolyte content. Neonates require 120 kcal/kg per day. Protein restriction should be avoided in neonates. When enteral nutrition is not feasible, it may be necessary to resort to parenteral nutrition. High concentrations of glucose need to be given to provide the required non-protein calories in the limited permissible volume necessitating central venous access.

125.7 Acute Renal Replacement Therapy

Acute renal replacement therapy (RRT) is indicated when the renal failure produces serious disturbances such as fluid overload, severe hyperkalemia, uncontrolled metabolic acidosis, hyperphosphatemia or hyponatremia unresponsive to fluid restriction [1, 3, 4]. Even in the absence of these indications, RRT may be required to create enough fluid space by ultrafiltration to enable administration of the necessary medications, blood products and nutrition that the child may need.

125.7.1 Peritoneal Dialysis

Peritoneal dialysis is the most common and widely used option for RRT in this age group [3, 4]. It is easily instituted even in the very low birthweight infant with either percutaneous bedside placement of a stiff catheter or surgical placement of a soft Tenchkoff catheter. Acutely ill babies on ventilator may not tolerate large intraperitoneal volumes. Small volumes with short dwelling-times may suffice to tackle the acute life threatening problems. Bicarbonate dialysis is needed in children with liver dysfunction or advanced sepsis as use of lactate-based dialysis fluid may lead to worsening of acidosis in these babies. The presence of intra-abdominal pathology may preclude the use of the peritoneum for dialysis.

125.7.2 Hemodialysis

Hemodialysis is infrequently used in this age group and can be considered only in hemodynamically stable babies. In neonates it requires the availability of suitably small dialysers and tubings. Blood or albumin priming of the lines and filter is usually needed. It can only be done in units where sufficient technical expertise is available.

125.7.3 Continuous Venovenous Hemofiltration

Continuous venovenous hemofiltration is a modality that can be used in sick neonates requiring renal replacement. It allows greater fluid removal, better hemodynamic stability and better nutrition. Its use in critically-ill children with renal failure is increasing steadily and 30% of centers surveyed in North America considered it as the preferential mode of treatment irrespective of age [34].

125.8 Outcome

Despite major advances in both neonatology and nephrology the mortality in neonates with ARF is fairly high and ranges from 45 to 60% [35]. Neonates with prerenal failure have a much better outcome than those with intrinsic renal failure. The worst outcome is seen in babies with intrinsic renal failure in the context of multiple organ dysfunction. As the de-

velopment of renal failure is a reflection of the severity of illness, it is not surprising that the mortality is so high. Babies with non-oliguric renal failure have a much better outcome than those with oligo-anuric renal failure [35].

Following recovery from the ARF, residual renal dysfunction may be seen in more than half the babies without an underlying congenital uropathy [21]. Incomplete recovery is invariable in those neonates who have suffered cortical necrosis. Although numerically not a large group, babies with *in utero* exposure to NSAIDs, ACEIs or ARB have a poor renal outcome and are likely to have persistent irreversible renal failure. Long-term follow-up of ELBW infants with neonatal renal failure has shown that nearly 45% of the children show a decrease in GFR over time. Urine proteins/creatine ratio > 70 g/mol creatinine (0.6 mg/mg creatinine) and serum creatinine > 55 μmol/L (6 mg/L) at 1 year of age were predictors of a poor prognosis for long-term renal function [36].

125.9 Present and Future Challenges

The challenges that lies before us are the following: a) to find markers that could detect renal injury much before it develops into renal failure; b) to find pharmacological measures that could prevent the development of renal failure in babies faced with stressful renal situations; c) to identify measures that would ameliorate renal failure and hasten renal recovery.

125.9.1 Early Diagnosis

Creatinine is an insensitive biomarker for GFR especially in the neonatal period and is blind to the early fall in GFR. More sensitive biomarkers are required that will facilitate early identification of renal injury. The hope that cystatin C could play such a role appears illusory [26]. The appearance of markers of renal tubular injury in the urine may precede the changes in GFR and may help in the diagnosis of acute kidney injury even before failure sets in. Biomarkers of renal injury such as NGAL, KIM-1 and IL-18 have been shown to appear in the urine more than 24 hours before the rise in serum creatinine and may serve as early diagnostic tools in the recognition of renal injury.

125.9.2 Prevention and Amelioration of Renal Failure

125.9.2.1 Animal Studies

Several pharmacological measures have shown varying degrees of success in preventing or ameliorating renal failure in experimental animals. Agents that favorably modulate renal hemodynamics as well as agents that counteract the mechanisms mediating cellular injury have been studied.

Protection of the renal hemodyamics has been attempted by the use of selective antagonists to endogenously produced vasoconstrictors or by the use of renal vasodilators. Selective antagonists that have been used with success include theophylline in hypoxia-induced renal failure and endothelin antagonists in sepsis. Atrial natriuretic peptide, a potent renal vasodilator, has also shown some promising results. Hypoxic ischemic insults to the kidney lead to renal tubular injury by cellular depletion of ATP as well as intracellular accumulations of calcium. Measures to restore cellular ATP by administration of ATP-$MgCl_2$ appear to ameliorate the hypoxemia- induced ARF in animals. Thyroxine also hastens cellular repair and renal recovery by restoration of cellular ATP and may prevent reoxygenation injury [2, 4]. Pretreatment with calcium channel blockers prevents the intracellular accumulation of calcium and the intense vasoconstriction that occurs with various renal insults such as ischemia and cyclosporine administration.

125.9.2.2 Human Studies

Many of the vasoactive agents have pronounced systemic effects or could potentially affect extrarenal organs and systems that preclude their routine clinical use until validated by human studies. Low-dose theophylline has shown promises when used to prevent renal failure in neonates undergoing perinatal asphyxia or respiratory distress syndrome [4, 26, 37–39]. In two small studies thyroxine has been used in neonates with hypoxemic renal failure with recovery of renal function. The possibility of using pharmacological measures to prevent renal failure needs to be systematically studied in humans before it can translate into standard clinical practice. The most important measures to prevent acute neonatal renal failure is to pay attention to general measures that include adequate hydration and maintenance of electrolyte and acid-base balance, and to limit the use of nephrotoxic drugs including NSAIDs in the hemodynamically-vulnerable perinatal period.

125.10 Chronic Renal Failure

125.10.1 Causes of Chronic Renal Failure in Newborn Infants

In certain circumstances, infants presenting with ARF do not recover completely and enter a state of chronic renal failure (CRF). Other babies present from the start with a chronic state of renal failure. The main neonatal causes leading to CRF are listed in Table 125.5. Prolonged ischemia can lead to irreversible tubular necrosis and cortical necrosis.

Table 125.5 Causes of chronic renal failure in the first year of life *

Cause	No of patients
Renal dysplasia	18 (37%)
Obstructive uropathy	16 (33%)
Polycystic kidney disease	5 (10%)
Congenital nephrotic syndrome	5 (10%)
VACTERL association	3 (6%)
Denys-Drash syndrome	1 (2%)
Unknown origin	1 (2%)
Antifungal agents (Fluconazole)	–
Cardiac drugs (Digoxin)	–

* Data from 49 infants. Reproduced from [40] with permission.

Congenital abnormalities of the kidneys and urinary tract are the most common causes of CRF, and include renal hypoplasia and cystic dysplasia, polycystic disease of the kidney, severe obstructive uropathy or reflux nephropathy, and posterior urethral valves [10, 21]. Vascular disorders such as renal vein or artery thrombosis, prolonged ischemia with subsequent irreversible tubular or cortical necrosis, disseminated intravascular coagulation and hemolytic disease with massive myoglobinuria represent acquired causes of CRF. Congenital forms (finnish type) of nephrotic syndrome can also induce early onset CRF.

125.10.2 Presentation

125.10.2.1 Malformative Uropathies

Antenatal ultrasound may reveal anomalies of the kidney, bladder and urinary tract that can permanently compromise postnatal renal function. Obstruction to urine flow by posterior urethral valves results in bilateral hydronephrosis and hydroureter, distended thick-walled bladder, dilated urethra and renal dysplasia [41]. The associated oligo-anuria and oligohydramnios may be the cause of marked pulmonary hypoplasia. Optimal management of posterior urethral valves requires a multidisciplinary approach by obstetricians, neonatologists, pediatric nephrologists and urologists.

After birth, the presence of a palpable abdominal mass or bladder may be the first clue to underlying CRF. Ultrasound examination usually leads to the diagnosis. The occurence of urinary tract infection is often the first clinical manifestation of congenital uropathies [10].

125.10.2.2 Failure to Thrive and Anemia

Failure to thrive, usually associated with poor and difficult feeding, is almost always present in infants with chronic renal failure [42]. Normochromic-normocytic anemia, secondary to the uremic state, is a manifestation of a decrease in erythropoietin formation by the sick kidneys.

125.10.2.3 Hypertension and Congestive Heart Failure

Arterial hypertension may have a renovascular origin, or be secondary to salt and water overload with or without congestive heart failure. Loop diuretics are indicated.

125.10.2.4 Nephrotic Syndrome

The presence of edema, heavy proteinuria and hypoalbuminemia at birth indicates the presence of a nephrotic syndrome, usually of the finnish type. The prognosis is extremely poor, the infants dying from sepsis or developing chronic renal failure in the first weeks of life.

125.10.3 Management of Chronic Renal Failure

125.10.3.1 Nutrition

Adequate nutritional therapy is essential to preserve the growth of the infant. An energy intake of 115 kcal/kg is necessary to sustain growth during the first 3 months of life, with appropriate calcium, phosphorus and sodium intakes. Many anorexic infants require calorie supplements (glucose polymers or fat emulsions) that often can only be administered by naso-gastric or gastrostomy tubings. The help of a skilled pediatric dietetician is mandatory to provide optimal nutrition in infants with CRF. When nutritional intervention fails to improve growth, treatment with growth hormone must be considered [43].

125.10.3.2 Fluid, Electrolyte and Acid-Base Balance

Most infants with CRF present with metabolic acidosis, hypocalcemia, hyperphosphatemia. These factors all inhibit growth. The acidosis must thus be compensated by the administration of sodium bicarbonate (2–3 mmol/kg per day), with the risk of inducing arterial hypertension; the hypocalcemia and the hyperphosphatemia by the administration of calcium carbonate (starting dose: 100 mg/kg per day). The levels of parathormone (PTH) may be increased in response to both hypocalcemia and hyperphosphatemia, the administration of calcium not being sufficient to prevent the occurrence of hyperparathyroidism and renal osteodystrophy. In this case, the administration of calcitriol (1,25-dihydroxycholecalciferol), orally or intraperitoneally, may be necessary [44].

125.10.3.3 Anemia

The anemia associated with CRF is due to inadequate production of erythropoietin by the kidney. Recombinant alpha or beta erythropoietin, given weekly or twice monthly by sc or iv injection, will correct the anemia.

125.10.3.4 Arterial Hypertension

When hypervolemia contributes to the hypertension, loop diuretics (furosemide, torasemide) or metozalone can be administered. ACE inhibitors or ARBs are particularly effective in renin-dependent hypertension. But drugs interfering with angiotensin II can induce a sharp decrease in GFR in volume-depleted patients because of an acute decrease in intraglomerular pressure. Calcium channel blockers are usually well-tolerated. The use of beta-blockers is limited because of the risk of symptomatic bradycardia [22].

125.10.4 Renal Replacement Therapy

Failure to normalize growth or to control the electrolyte disturbances is an indication to start dialysis. In neonates and infants, peritoneal dialysis is the method of choice [45]. It is easy to perform, and has been shown to be effective. It can be used until the child can undergo renal transplantation. Peritoneal dialysis can be performed manually with 4–5 dialysis fluid exchanges a day. When available it is best performed using an automated cycling machine that can deliver 8–10 exchanges over 8–10 hours. Although providing effective renal replacement therapy [46], peritoneal dialysis in infants carries a significant risk of infection and death, with repeated episodes of peritonitis. For parents, the burden of long-term peritoneal treatment in infants is substantial [32, 47].

No neonate with CRF should be started on RRT unless there is a hope for later kidney transplantation. Full immunization should be completed before considering transplantation, usually only after a body weight of 8–10 kg, and an age of 2–3 years are attained.

125.11 Conclusions

The morbidity rate of ARF in newborn infants is elevated. In those developing CRF, RRT is possible with relative success. Decisions concerning RRT must be discussed with the family and an expert ethical committee, keeping in mind the long-term outcome of severe CRF in very young children. In developed countries this outcome is globally as follows: one third of the infants die, half have a functioning renal graft and the rest will have returned to dialysis in the first years of life [32].

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Diagnosis and Treatment of Renal and Urinary Tract Malformations

Vassilios Fanos, Marco Zaffanello and Michele Mussap

126.1 Introduction

Renal and urinary tract malformations in newborns are mostly congenital anomalies with genetic bases. The routine of antenatal ultrasound (US) scans has resulted in the early detection of these conditions and in selected cases has leaded to the development of prenatal management strategies including fetal intervention and/or the organization of the diagnostic procedures, postnatal surgical intervention and/or clinical follow-up. In minor cases, where diagnosis is not allowed during prenatal life, it may be obtained after a postnatal routine follow-up or subsequently a clinical complication, generally urinary tract infection (UTI).

126.2 Etiology and Pathogenesis

The development of the kidney results from the interaction between the ureteral bud and the metanephrogenic mesoderm in the intermediate mesodermal layer. At the fifth week of embryonic development, the ureteral bud grows and comes in close contact with mesodermal elements. The ureteral bud undergoes a series of divisions, which give rise to the pelvicalyceal system. At the same time, mesoderm differentiates to form the nephrons, a process that is completed by 36 weeks of gestation. During intrauterine life, the placenta provides the main source of fluid and electrolyte regulation. Therefore, urine secreted by the fetal kidney provides the main volume of the amniotic fluid [1].

Several genes may have roles in multiple stages of kidney and lower urinary tract morphogenesis. Specific mutations of genes involved in renal tract development have been found in individuals with familial occurrence.

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126.2.1 Genetic Factors

A spectrum of renal tract malformations is part of the genetically defined syndromes with Mendelian distribution of inheritance that could be classified as syndromic and nonsyndromic urinary tract malformations.

126.2.1.1 Syndromic Renal and Urinary Tract Malformations

Edith Potter subdivided cystic dysplastic kidney (CDKs) into those with little normal tissue, which she considered to arise from a primary defect in interaction and induction between the ureteric bud and metanephric mesenchyme versus others, in which nephrogenesis had begun, but then become interrupted. Many corresponding genes are known to be expressed in development of the human renal tract, with some of these genes recently found to be mutated in individuals with either isolated (non-syndromic) kidney malformations or renal tract

Table 126.1 Syndromes associated with renal and urinary tract malformations

Autosomal dominant syndromes
1. Branchio-oto-renal [4]
2. Hypoparathyroidism, sensorineural deafness, and renal dysplasia [5]
3. Mayer–Rokitansky–Küster–Hauser [6]
4. Renal coloboma [7]
5. Renal cysts and diabetes [8]
6. Townes–Brocks [9]

Autosomal recessive syndromes
1. Bardet–Biedl [10, 11]
2. Fraser [12, 13]
3. Jeune [14]
4. Meckel–Gruber [15]
5. Prune belly syndrome [16]

X-linked syndromes
1. Kallmann [17]
2. Simpson–Golabi–Behmel [18]

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

malformations associated (syndromic) with defects in other organ systems [2]. Thus, multiorgan syndromes associating with a renal tract malformation may be autosomal dominant, autosomal recessive or X-linked [3]. Each syndrome may associate a large spectrum of renal tract malformations with the involvement of different organs (Table 126.1).

126.2.1.2 Nonsyndromic Renal and Urinary Tract Malformations

Common renal malformations encountered in the fetal period are hydronephrosis associated with primary vesicoureteral reflux (VUR) and duplex kidneys. These malformations may have genetic bases because they sometimes affect more than one family member, perhaps with two or more abnormal genes necessary in any one person to produce the anatomical change [19]. Analysis of data from humans and mice suggests that some of the renal damage associated with VUR is congenital and is due to a kidney malformation, not to secondary VUR/UTI related damage [20]. Human nonsyndromic renal tract malformations may be of both sporadic pattern and inherited fashion, dominant and recessive inheritance.

Half a decade ago the key anatomical differences between cystic dysplastic kidney (CDK) malformations and congenital polycystic kidney disease (PKD) were defined for the first time, with the identification of genes inherited with dominant (ADPKD) or recessive (ARPKD) fashion. However, dominant inheritance has been described for primary vesicoureteral reflux (VUR) [21], dysplastic or hypoplastic kidneys [22] and pyeloureteral junction obstruction (PUJO) [22]. Primary VUR and nephropathy have been described in few patients with *UPKIIIA* [23] and *ROBO2* genes mutations [19].

Dominant mutations of the paired box 2 (*PAX2*) gene, which is expressed in developing fetal kidney tubules, should be considered in individuals with renal hypoplasia or dysplasia, who have either visual impairment, from associated optic nerve coloboma and/or a similarly affected first degree relative [7, 24]. A polymorphism of the transcription factor *PAX2* is associated with reduced neonatal kidney size [25, 26]. Moreover, the *PAX2* gene, which is expressed in developing fetal kidney tubules, should be considered in individuals with renal hypoplasia or dysplasia, who have either visual impairment (from associated optic nerve coloboma) and/or a similarly affected first-degree relative in Renal-coloboma syndrome (RCS), an autosomal dominant disorder [2].

The main cause of fetal bilateral hyperechogenic kidneys is associated with hepatocyte nuclear factor 1-β (*HNF1B*) gene mutations [27]. Mutations of the gene that encodes the transcription factor *HNF1B* are associated with renal cysts and diabetes (RCAD) syndrome. Moreover, *HNF1B* mutations lead to different types of renal tract malformations, including multicystic dysplastic kidney (MDK), congenital solitary functioning kidneys (CSFK), cystic dysplastic kidneys, hypoplastic kidneys and glomerulocystic kidneys [8, 24, 27, 28]. *HNF1B* mutations or variants may be found in 8% of children who have dysplastic/hypoplastic kidneys and renal failure and in 22% who have cysts [24, 28, 29]. However, intronic regulatory expression sequences have been identified in the *HNF1B* gene [30] named retinoic acid regulatory element (*RARE*) and *T-MARE* (MafB Responsive Element) which links retinoic acid (RA) and *MafB* pathways with *HNF1B* gene regulation. Both *MafB*, a member of the large Maf family of transcription factors, and the RA signaling pathway have been implicated in renal development. In particular, *MafB* is specifically expressed in glomerular epithelial cells (podocytes) and is essential for podocyte differentiation and renal tubule survival [31]. However, RA signaling modulates the expression of renal transcription factors and components of signaling pathways that are known to direct segment fates during mammalian nephrogenesis.

Several other genes are identified since involved in aberrant renal and urinary tract morphogenesis. The *c-ret* gene encodes an epithelial cell, trans-membrane tyrosine kinase receptor protein. In *c-ret* mutants, renal agenesis, severe hypodysplasia and blind-ending ureters are observed [25]. Two X-linked gene mutations, angiotensin type II receptor gene (*Agtr2*) [32, 33] and L1-cell adhesion molecule (*L1-CAM*) are involved in PUJO or MDK, and in the process of kidney and urogenital tract organogenesis [25]. Bone morphogenic protein 4 (BMP4) has also been shown to interact with proteins in systems outside of the kidney and urinary tract that have been identified as regulators of kidney and urinary tract organogenesis. WNT11 is a target molecule of glial cell line-derived neurotrophic factor (GDNF) and down regulation by BMP4 results in the inhibition of ectopic ureteric bud formation from the Wolffian duct. The *Foxc1 C*/*Foxc2 C* compound heterozygotes have hypoplastic kidneys and single ureters. However, *Uroplakin* genes may be critical for development of both the upper and lower urinary tract in humans. De-novo heterozygous mutations in *Uroplakin IIIA* are associated with severe renal adysplasia and hypodysplasia with kidney failure in childhood [34]. Furthermore, mutations of genes encoding Renin-Angiotensin System (RAS) components are associated with perinatal kidney disease. The renal phenotype usually comprises slightly large kidneys, appearing bright on fetal ultrasound, together with a profound decrease in amniotic fluid volume [35]. Finally, evidence is emerging that posterior urethral valves (PUVs) may have a genetic basis, possibly a male-limited autosomal recessive condition: the exact genes involved, however, are currently unknown [2].

Considerable recent progress has been made in unraveling the rather complex genetic bases of the spectrum of diseases known as the Bardete-Biedl, Meckele-Gruber and Joubert syndromes. These syndromes show considerable phenotypic overlap, with all featuring perinatal kidney disease with dysplasia and cysts [2].

126.2.2 Environmental Factors

Some observational studies linked the environmental factors with the development of malformation in renal and urinary tract in the fetus and newborn. Renal tract malformations are more common in fetuses of mothers with high alcohol intake or diabetes mellitus [3]. However, gross gestational insults, such as twin–twin transfusion syndrome and heavy prematurity, may perturb human kidney differentiation [36, 37]. Moreover, the discovery of mutations in genes coding for the RAS cascade in patients with renal tubular dysgenesis [35] are associated with the increased incidence of renal anomalies in fetuses exposed to drugs that block angiotensin II signaling [38].

126.3 Clinical Aspects

126.3.1 Prevalence

Prenatal ultrasound (US) screening programs assessing the diagnostic prevalence of renal anomalies in 20 registries of 12 European countries identified unilateral MDK in 1:7100 of fetuses, unilateral renal agenesis in 1:12,500, bilateral renal agenesis or dysplastic kidneys in 1:7700 and PUVs in 1:33,300. Terminations of pregnancies were performed in 67% of the detected bilateral renal agenesis/dysgenesis, but only 4% of the unilateral MDK malformations [39].

The US screening of fetuses in the last trimester showed that 7.7% had transient upper renal tract dilatation [40, 41]. From a multicenter study, prenatal hydronephrosis had an overall prevalence of 11.5 cases per 10,000 births. The large majority of cases were live births (96% of total), only a minority were terminations of pregnancy or fetal deaths. Boys accounted for 72% of all cases. A high proportion of the cases (86%) had an isolated renal tract malformation [42]. Hydronephrosis may be bilateral in 37–57% of cases [43]. Among hydronephrosis, PUJO was the most common cause of prenatal pelvis dilatation, accounting for 33% of infant cases, followed by primary VUR (28%), megaureter (18%) and complicated renal duplex kidney (12%). Less frequent conditions of prenatal renal pelvis dilatation were MDK, PUVs, hydronephrotic horseshoe kidneys, and hereditary renal adysplasia [44]. MDK, although not classified as hydronephrosis, may be mistaken for hydronephrosis [45].

Sometimes the hyperechogenic kidney, detected with prenatal US, may pose problems of differential diagnosis. The prevalence of renal pathology associated with kidney hyperechogenicity at US detected after 17 weeks' gestation has been assessed in a retrospective multicenter study. The conditions more frequently associated with fetal hyperechogenic kidneys were ADPKD and ARPKD. Less frequent conditions were Bardet-Biedl syndrome, Meckel-Gruber syndrome and Ivemark II syndrome. Jarcho-Levin syndrome, Beemer syndrome and Meckel-like syndrome were rare. Lastly, one third of the fetuses with hyperechogenic kidneys had renal cysts [46].

126.3.2 Classification of Renal Anomalies

An abnormal human renal tract may develop from the beginning of the metanephros to the end of nephron formation, which occurs at about 34 weeks of gestation [47]. The acronym CAKUT means congenital anomalies of the kidney and urinary tract, a syndrome that includes a wide spectrum of anomalies affecting the formation of the kidney and bladder. In the kidney, these anomalies include hypoplasia, dysplasia or a complete lack of kidney formation (agenesis). Proximally the ureter can be obstructed (PUJO) or grossly dilated (megaureter). Distally the ureter can be blocked at its insertion into the bladder (vesicoureteral junction obstruction [VUJO]). Alternatively, the ureter may be incompetent (VUR) or insert outside the bladder (ectopic). Bladder and urethral anomalies include abnormal hemitrigone formation as well as urethral valves (PUVs) and urethral atresia [25].

The anomalies that affect the renal system may be classified according to the renal and urinary tract district involved (Table 126.2) [1].

Table 126.2 Classification of main anomalies affecting the renal system

- Renal agenesis
  - Unilateral
  - Bilateral (Potter's syndrome)
- Abnormalities of position (ectopia)
- Kidney fusion
  - Horseshoe kidney
  - Crossed fused ectopia
- Dysplasia/cystic renal disease
  - Aplastic or tiny dysplastic remnant kidney
  - Hypoplastic kidney
  - Simple renal cysts
  - Multilocular cysts
  - Multicystic dysplastic kidney (MDK)
  - Polycystic kidney disease (ADPKD and ARPKD)
- Obstructive uropathy
  - Posterior urethral valves (PUVs)
  - Pelviureteric junction obstruction (PUJO)
  - Vesicoureteric junction obstruction (VUJO)
- Vesicoureteral reflux (VUR)
- Ureteral alterations, duplications and ectopia
  - Ectopic ureter
  - Ureterocele
  - Ureteral duplications
- Anomalies of the bladder
  - Epispadias
  - Classical bladder exstrophy
  - Bladder exstrophy complex
- Anomalies of the penis

Modified from [1].

126.3.3 Renal Agenesis

Embryologically, renal agenesis occurs because the ureteric bud has not interacted with the metanephric mesenchyme. The bud fails to form the ureter, renal pelvis and collecting ducts. The mesenchyme fails to form nephrons. On occasion, unilateral renal agenesis is accompanied by genital tract anomalies on the same side [48]. The most profound and severe renal tract malformation is the complete bilateral kidney development, called renal agenesis, oligohydramnios sequence or Potter's syndrome, often accompanied by absent ureters. Prenatal diagnosis of oligohydramnios in the second trimester is dependent on US scanning and often a full post mortem examination is necessary to identify any underlying fetal cause [49]. Bilateral renal agenesis or hypoplasia was associated with a mother's risk factors such as adiposity prior to pregnancy, smoking during the periconceptional period, and binge drinking during the second month of pregnancy [50].

126.3.3.1 Diagnosis

Renal agenesis is suspected when US does not identify the renal parenchyma and a postnatal scintigraphic scan does not show any renal function in a given side of the body. A nonfunctional kidney is diagnosed when US identifies residual renal parenchyma, without valuable scintigraphic renal function (Fig. 126.1).

126.3.3.2 Management of Bilateral Renal Agenesis

The diagnosis of bilateral renal agenesis is made during pregnancy by US scan, which show the absence of the kidneys and non-visualization of the bladder. Since this syndrome (Potter's syndrome) is frequently associated with poorer life expectancy, termination of the pregnancy is the most frequent outcome.

126.3.3.3 Management of Unilateral Renal Agenesis

Diagnosis of unilateral renal agenesis is generally performed by prenatal US. Controlateral compensatory renal hypertrophy can be detected in the third trimester and occasionally in the latter part of second trimester [51].

Postnatal radionuclide scan (DMSA) may confirm the diagnosis and establish the functional state of the CSFK. After the diagnosis of unilateral renal agenesis, postnatal screening is mandatory to rule out other associated renal anomalies. Early diagnosis and treatment of urological anomalies associated with CSFK is imperative to decrease the long-term risk of renal damage [52]. The early identification of risk factors for renal damage is essential because CSFK may further exhibit growth failure even if it was initially normal [53]. The management of VUR in CSFK follows the same strategies used to manage unilateral VUR in children with two normally functioning kidneys [54]. On the other hand, infants with PUJO in CSFK who have severe pelvis dilatation and decreased renal function require early surgical therapy or selective internal ureteric stenting [55]. Severe renal hypodysplasia, mistaken for renal aplasia, may evolve into renal malignancy and may cause hypertension.

126.3.3.4 Prognosis

CSFK may progressively lead to persistent hyperfiltration, microalbuminuria and increasing proteinuria, with focal and segmentary hyalinization of the renal glomeruli requiring periodical long-term follow-up.

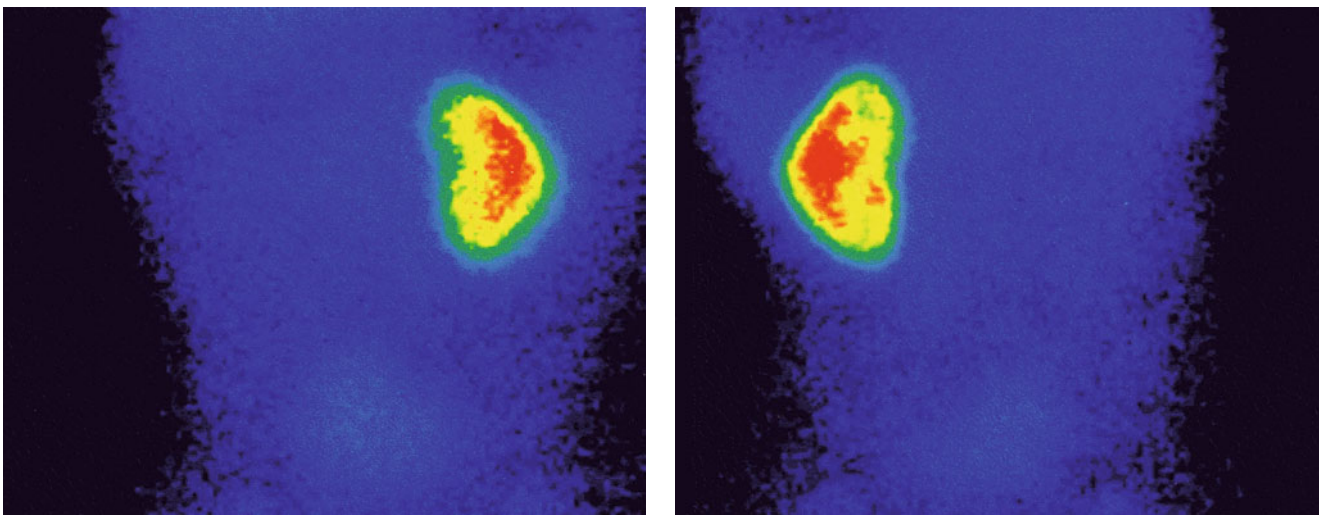

Fig. 126.1 Renal DMSA scintigraphy performed in a male-baby with left solitary hypertrophic functioning kidney and right iliac-sided ectopic not-functioning kidney. Reproduced from [53] with permission

126.3.4 Abnormalities of Position

During embryological development, the kidney undergoes rotation as well as cranial migration taking its blood supply from the middle sacral artery, iliac artery and the aorta. Malrotation may occur as result of incomplete, excessive or reversed rotation. In these situations, the renal hilum faces in an abnormal direction and gives rise to obstruction. Anomalies of ascent may result in abnormal positions of the kidney of which the most common is the pelvic kidney. The abnormal location frequently predisposes to UTI and VUR [1].

126.3.4.1 Management

Asymptomatic patients, with adequate renal function, require inclusion into follow-up protocols. Imaging with US and radionuclide scan (DMSA) is required to identify location and associated abnormalities. Overall, a radionuclide scans is advisable when the pelvic position makes the kidney difficult to identify by US. Computed tomography/magnetic resonance imaging (CT/MRI) scan is advisable in cases where renal function is poor. Voiding cystoureterogram (VCUG) or scintigraphic screening may rule out a concomitant VUR.

Adequate renal function associated with clinical and radiological evidence of obstruction or VUR may require surgical consideration. Not functioning, poorly functioning, and MDK associating obstruction or recurrent infections are normally treated by nephrectomy.

126.3.5 Kidney Fusion

In crossed fused ectopia, the upper pole of one kidney is fused with the lower pole of the opposite kidney. Crossed renal ectopia is most frequently associated with VUR and UTI. Fusion of renal elements may occur across the midline to produce a horseshoe kidney (Fig. 126.2), which is one of the most frequent genitourinary abnormalities with an overall incidence of 1/666 [56]. Renal fusion anomalies may be asymptomatic but are frequently associated with obstruction and infection. In a horseshoe kidney, the most common clinical problem is obstruction at the pelviureteric junction. Horseshoe kidney may be part of the spectrum of well-known syndromes associated anomalies affecting multiple organs such as Turner's, Frasier's, Renal Coloboma and Mayer-Rokitanski.

A recent survey reports that concomitant urogenital and non-urogenital malformations were equally frequent for horseshoe kidney and crossed fused ectopia. Severe anomalies or malformations were found in 23% of patients, less frequent than previously reported [57].

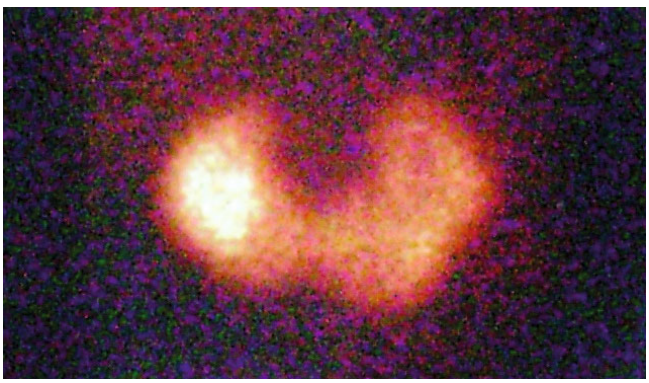

Fig. 126.2 Anterior view of DMSA scintigraphy in a baby with horseshoe kidney

126.3.5.1 Management

The diagnosis of fusion abnormalities can be made with the commonly used imaging modalities, such as US and scintigraphy. In cases of obstruction confirmed by radionuclide scan (MAG3), a pyeloplasty is commonly required. Patients with VUR, demonstrated by VCUG, requires surgery if UTIs are frequent. Individual cases of complex anatomical situations require special examination strategies, and CT appears to be the most reliable imaging method [57]. Finally, parenchyma with poor renal function or dysplasia associated with recurrent infections may need partial nephroureterectomy.

126.3.6 Renal Dysplasia

Dysplastic kidneys are so-called because the both organs are present but development is abnormal and incomplete. These organs contain incompletely branched ducts derived from cells of the ureteric bud and collecting duct line, surrounded by undifferentiated and metaplastic stroma. The classic antenatal presentation of dysplastic kidneys is of large bright kidneys, with or without cystic spaces, occurring within the cortex at the routine 20-week scan [34].

126.3.6.1 Aplastic or Dysplastic Kidney

The dysplastic kidney may be either aplastic (a few millimeters long) or large and distended by multiple cysts. Ureters attached to MDKs are scarcely formed and with non-patent sections. These severe types of dysplastic kidneys generally have no excretory function.

126.3.6.2 Hypoplastic Kidney

Hypoplastic kidneys contain fully formed nephrons but have a deficit in nephron number. Kidney hypoplasia is defined as

kidney mass below 2 standard deviations of that of age-matched normal individuals or a combined estimated kidney mass of less than half of normal for the patient's age. Oligomeganephronia represents a severe variant of hypoplasia in which both kidneys are one-eighth to one-half normal weight. Nephron number is reduced by 80% and nephrons are markedly hypertrophied, with glomerular diameter more than twice normal. Premature babies and/or infants small for gestational age due to intrauterine malnutrition will be born with relatively small kidneys and a certain nephron deficit, a condition called congenital oligonephropathy [58].

126.3.6.3 Cystic Dysplastic Kidney

In dysplastic kidneys, cysts arise in the context of abnormal renal development. Cystic dysplastic kidney contains primitive tubules and cysts alongside normal nephrons and generally may have excretory function. Renal tubular dysgenesis is related to incomplete differentiation of proximal tubular nephron segments, often associated with fetal anuria and oligohydramnios. In the variant of kidney malformation associated with obstruction, the first filtering nephrons develop normally, but nephrogenesis subsequently halts developing subcapsular cysts from nephron precursors [59, 60]. Moreover, dysplasia is sometimes limited to the medulla and, in the presence of a duplex ureter, only the upper moiety is generally dysplastic.

126.3.6.4 Multicystic Dysplastic Kidney

Histology of fetal MDK showed that few nephrons, containing relatively normal glomeruli and proximal tubules, are present in these kidneys [61]. MDK result from complete obstruction early in embryonic development. Abnormal structures seem to be destroyed later in gestation or postnatally [62]. In unilateral MDK, the presence of minimal function is rare (3–7%), occurring in 4% cases of patients [63].

Management and Prognosis

In asymptomatic patients, a follow-up with serial US scans is mandatory. US screening may detect spontaneous involution in the most severely affected kidney. All MDK patients on conservative management will need regular scans until at least 5 years of age.

Symptomatic patients with increasing abdominal distension and poor feeding may require nephrectomy [64]. Surgeons advocated prophylactic removal of these asymptomatic lesions for possible risks of hypertension and malignancy. The risk of hypertension is less than 1%. If severe hypertension is detected it will be necessary to carry out curative nephrectomy [65]. However, malignancy has not been found [64]. If neoplasms occur, they usually present as Wilms' tumor. If any increase in size or unusual features appears this will be an indication to proceed to nephrectomy.

Bilateral involvement leads to lethal pulmonary hypopasia from anhydramnios.

126.3.6.5 Polycystic Kidney Disease

Polycystic kidney disease (PKD) is a genetic disorder presenting diverse forms:

- Autosomal recessive polycystic kidney disease (ARPKD)
- Autosomal dominant polycystic kidney disease (ADPKD)
- Nephronophthisis/medullary cystic kidney disease

PKD is a disease of the nephron with multiple renal tubular cysts that leads ultimately to end stage renal failure (ESRF) [66]. It has been estimated that there are approximately 6.5 million people in the world with inherited PKD [67]. Inherited PKD may have either dominant or recessive inherited fashion. The dominant form (ADPKD) is due to mutations in two different genes, *PKD1* or *PKD2*. The protein products of the PKD genes, polycystin-1 and polycystin-2, regulate renal tubule epithelial cell division and differentiation. The recessive form (ARPKD) and nephronophthisis/medullary cystic kidney disease are associated with the *PKHD1* and *NPH1* genes and their protein products are fibrocystin and nephrocystin, respectively.

Nephronophthisis or medullary cystic kidney disease was associated in one fourth of cases with Joubert syndrome, an autosomal recessive disorder that is described in patients with cerebellar ataxia, mental retardation, hypotonia, and neonatal respiratory dysregulation [68].

ARPKD is an inherited recessive disorder in which both kidneys are affected by cysts developing from dilated collecting ducts. The condition is associated with congenital hepatic fibrosis leading to the development of portal hypertension. The renal cystic changes appear in utero, and are associated with poor renal function leading to oligohydramnios. Neonates with severe disease have a high mortality up to 50%. Severe kidney disease tends to be associated with milder liver involvement, whereas milder renal disease applies when liver manifestations are predominant. Differential diagnosis is made on the history, clinical examination, prenatal and postnatal imaging by US. In some cases, biopsy of the kidney and liver may be necessary to confirm the diagnosis. Many families who lost a child with severe ARPKD desire an early and reliable prenatal diagnosis. Given the limitations of antenatal US, this is only feasible by molecular genetics that became possible from 1994 when *PKHD1* was mapped [69].

Management

Patients require evaluation of renal function providing appropriate supportive measures if renal failure occurs, treating hypertension and metabolic disturbances. If kidneys are massively enlarged causing respiratory difficulty a nephrectomy may be required with either unilateral or bilateral and dialysis.

126.3.7 Obstructive Uropathy

Prenatal suspicious of obstructive uropathy at US may be made as marked dilatation of the urinary tract. Antenatal pelvicalyceal dilatation has been reported in ~0.6% of pregnancies. Fetal lower urinary tract obstruction affects 2.2 per 10,000 births [70].

Many different causes are involved in the pelvicalyceal dilatation, including transient hydronephrosis, physiological hydronephrosis, PUJO and PUVs [71]. Obstructive uropathy can be classified as lower or higher urinary tract obstruction. High urinary tract obstruction is suggested when there is hydronephrosis or pelvicalyceal dilatation in the absence of visible ureteral bladder pathology, either at prenatal or postnatal assessment. The most common lower anomaly is PUVs, which accounts for approximately half of cases, followed by urethral obstruction/atresia [70]. Congenital megaloureter is a poorly defined entity in which ureterectasias is associated with no reflux and normal bladder [72]. Many newborns with pelvicalyceal dilatation can be safely treated non-operatively and obstruction occurs in only small number of patients. In a series of patients, 54% of cases the hydronephrosis improve or resolved spontaneously during the first 3 years of life [73].

126.3.7.1 Posterior Urethral Valves

PUVs is a congenital disease of high mortality and morbidity, starting in the uterus and leading to progressive renal dysfunction. PUVs are recognized as the most common cause of bladder outlet obstruction in boys with an incidence of 1 in 2,000–4,000 live male births [1]. Commonly, PUVs consist of two folds arising from the urethra at the level of the veru montanum and fusing anteriorly in the midline. A much rarer form is a diaphragm extending across the lumen of the urethra just beyond the veru montanum. The degree of obstruction can vary from minimal with associated symptoms of dysfunctional voiding to severe with dilated posterior urethra, trabeculated bladder and severely hydronephrotic kidneys.

Antenatal Diagnosis

The antenatal diagnosis is made on the finding of dilated upper tracts associated with a thick-walled bladder and oligohydramnios. The bladder appears thick-walled and tense in cases of obstruction whereas in prune belly syndrome the bladder appears floppy [74]. A detailed sonographic evaluation of the fetus is carried out to exclude extrarenal abnormalities. MRI is reported of benefit when used in addition to US if the resolution of US is impaired by oligohydramnios [75].

Antenatal Management

Prenatal diagnosis provides the opportunity to improve functional outcome by either decompression of the obstructed bladder in intrauterine life or prompt postnatal intervention to relieve obstruction, minimize the risk of sepsis and optimize management of the valves [65]. Fetal intervention is not without risk of complications and should be avoided where renal function is poor with little possibility of survival. However, fetal intervention is not indicated in patients detected prior to 24 weeks because the renal changes are not reversible. Where the diagnosis is made after 24 weeks, the outcome in terms of renal function is generally favorable [76]. Thus, fetal intervention should not be performed if the US indicates marked renal dysplasia or in cases with adequate renal function with stable amniotic fluid volume. Fetal intervention has conventionally been restricted to male fetuses with suspected PUVs, good renal prognosis and oligohydramnios. The procedure most commonly performed is percutaneous vesico-amniotic shunt [74]. In utero percutaneous vesico-amniotic drainage possibly improves overall perinatal survival as compared to the non-drainage group [77]. On the other hand, open fetal vesicostomy has a high risk of complications since risk for preterm labour, maternal morbidity, premature ruptures of the membranes and fetal death.

Postnatal Management

Open or endoscopic procedures would provide a definitive treatment of obstruction, although limitations are the uncertainty whether renal function can be significantly improved [78]. Anyway, some cases of PUVs may benefit from timely identification and surgical intervention, although prenatal diagnosis has little impact on mortality or ESRF in the first 10 years of life ,reflecting the crucial role of renal dysplasia as a determinant of early renal impairment [65].

Surgical Treatment

The initial management is to pass a urethral catheter into the bladder and establish drainage. If a urethral catheter cannot be passed because the bladder may contract around the urethral catheter, suprapubic drainage is required. Moreover, if contrast study of bladder and urethra visualizes the urethra adequately, the diagnosis of PUV will be confirmed and VUR if present will also be identified.

Primary valve ablation is performed transurethrally with miniature endoscopes and under direct vision; the coagulating electrode fulgurates the valve leaflets. There is good evidence that early valve ablation can lead to recovery of bladder function and improvement in the upper tracts [79]. Vesicostomy is generally reserved for neonates with severe upper tract changes and poor renal function especially if associated VUR. The temporary decompression of the bladder may help to improve upper tract function. Closure of the vesicostomy is carried out after valve ablation and when upper tract surgical intervention has been completed [80, 81]. In a long-term study, primary valve ablation gave the best results

with good capacity, compliant bladders similar to those with ureterostomy, while the vesicostomy group had small-capacity and hyperreflexic bladders [82]. From another study, the long-term results of prenatally detected PUVs confirm that early valve ablation can be considered as the primary treatment in the majority of patients, without the need for preoperative drainage or diversion [83].

Prognosis

Cases where the US features of PUVs appear early (before 20 weeks of gestational age) and upper tract dilatation is moderate to severe have a poor prognosis [84]. The kidneys are often dysplastic and have poor function. Many of these fetuses would either abort or be stillborn. In appropriately selected fetuses, intervention may improve perinatal survival, but long-term renal morbidity amongst survivors remains problematic. An important prognostic feature on US is the presence of significant oligohydramnios, which if present prior to 24 weeks is associated with a higher prevalence of pulmonary hypoplasia [76]. Moreover, both gestational age at diagnosis and oligohydramnios were statistically significant predictors of final renal outcome [83]. Finally, poor renal function associated with renal dysplasia could not improve even after relief of obstruction. Thus, a high percentage of cases progress to renal failure regardless of prenatal diagnosis [65].

126.3.7.2 Pelviureteric Junction Obstruction

Hydronephrosis secondary to PUJO is the commonest anomaly affecting the renal collecting system. On total of the antenatally diagnosed renal pelvis dilatation, PUJO occurred in 13% of cases [44]. Of total fetal hydronephrosis, PUJO accounted for 0.2–0.4% of cases, of which 20–25% of cases were bilateral [71].

Pathogenesis

The condition results from an intrinsic stenosis of the ureter at the point where it joins the pelvis. PUJO is characterized by aberrant pyeloureteric smooth muscle, which typically exhibits hypertrophy and perifascicular fibrosis and abnormal innervation [85]. The abnormal ureteric segment has decreased peristaltic activity resulting in failure of transport of urine from pelvis down to the ureter.

Clinical Presentation

Renal pelvis dilatations are frequently detected with prenatal US. Pelvicalyceal dilatation in the fetus must be interpreted with caution as many of these resolve spontaneously in the postnatal period. A system of grading based on the anteroposterior diameter (APD) of the renal pelvis is used to evaluate the severity of the hydronephrosis. Measurements of 3 mm or less are considered normal at any gestational age. Beyond 30 weeks' gestation, thresholds for diagnosis of mild, moderate, and severe hydronephrosis were defined as 5–8 mm, 9–15 mm, and greater than 15 mm, respectively. Most agree that postnatal evaluation is prudent if the renal pelvis APD reaches 10 mm at any point in gestation, or if calyceal dilation is noted. Recent prospective studies have found that even mild hydronephrosis that is stable or resolves over a pregnancy may redevelop, progress, and necessitate surgery after birth [72].

Management

The management of hydronephrosis secondary to PUJO is aimed at preserving long-term renal function. In infants, asymptomatic unilateral hydronephrosis with stable differential renal function can be managed conservatively while obstructive dilatation requires serial assessments and early surgical correction [86]. Ensuring a sufficient follow-up interval, especially during the first 3 years of life, is essential to prevent permanent loss of renal function in kidneys that do develop signs of obstruction [73].

All patients with prenatally detected hydronephrosis should have US scans, MAG-3 renograms and VCUG (to rule out VUR). On the US scan, APD of the renal pelvis, degree of caliectasis, renal length, and thickness of the renal cortex, echogenicity and cortico-medullary differentiation should be assessed.

The concept of measuring renal pelvic dilatation on US allows children to be categorized into a conservatively managed group (APD below 12 mm) and an early surgical intervention group (APDN 40 mm). If APD is below 12 mm these patients are not at risk and are followed up with US scans at 3 months and 1 year. When APD is from 12 to 20 mm these patients require US scans at 3 months and 1 year and MAG-3 scan at 3 months. If APD is from 20 to 40 mm these patients requires MAG-3 scan at age 1 month [87]. MAG-3 renogram provides a more accurate assessment of obstruction. On diuretic MAG3 scintigraphy, infants show renal pelvis dilatation with delayed drainage and relatively reduced renal function [88].

Fetal Intervention

Hydronephrosis with normal renal parenchyma is generally associated with good renal function and hence does not require fetal intervention. Fetal intervention should be considered in cases of severe bilateral hydronephrosis associated with oligohydramnios, bilateral hydronephrosis with dysplastic renal parenchyma and solitary kidney with severe hydronephrosis. Therefore, the risk of chronic or ESRF posed by upper tract obstruction is exceptionally low, except in the rare instances when it affects a solitary kidney [65].

Postnatal Management and Prognosis

Close follow-up is required in the first 2 years of life to identify the subgroup (35%) of children with obstruction that requires prompt surgery. Nonoperative management with close follow-up during the first 2 years is a safe and recommended approach for neonates with primary bilateral PUJO type hydronephrosis [73]. Large numbers of children have been submitted to an unnecessary pyeloplasty for asymptomatic PUJO, which was otherwise destined to resolve spontaneously [65]. However, from 20 to 40 mm with function near 40% these patients need close follow-up, by US and MAG-3 scan at 3 months, 6 months and 1 year. If the APD is near 40 mm, surgical intervention is required. Treatment and follow-up protocol for UPJO hydronephrosis was proposed by Onen et al [73] (Table 126.3).

Pyeloplasty of renal units with PUJO and decline of kidney function is widely suggested to improve renal outcome or delay long-term morbidity [89]. Such an approach prevented permanent loss of renal function. Actually, unilateral PUJO is less indicated for surgery, especially if renal function is unaffected. During follow-up, the main indications for surgery are deterioration of renal function of 5% or more, glomerular filtration rate drop to 40% or less, bilateral hydronephrosis or solitary kidney [90]. Generally, amelioration has been related to kidney maturation rather than a result of surgery [91]. If renal function is already reduced, the operation is made even if there is no proof of improving final renal outcome, as usually split renal function does not change. When renal function is poor at the beginning despite the pyeloplasty, the functional amelioration of the operated kidney is frequently absent or minimal [92]. In terms of relieving obstruction, a pyeloplasty has a success rate in excess of 95%. In the small percentage of patients requiring repeat pyeloplasty, the failure of the original procedure is usually apparent within a year or two of the original procedure [65].

Prenatal diagnosis and surgical intervention have contributed to a reduction in long-term morbidity in children with PUJO. Therefore, many children have almost certainly undergone unnecessary early pyeloplasty for an obstruction that would have resolved spontaneously [65].

Table 126.3 Treatment and follow-up protocol for ureteropelvic junction hydronephrosis

Grade 1
- Follow-up with serial ultrasound starting at 1 month
- No prophylactic antimicrobial
- No renal scintigraphy
- Follow-up for development of urinary symptoms

Grade 2
- Follow-up with ultrasound plus renal scintigraphy starting at 1 month
- Antibacterial prophylaxis may be beneficial
- Renal scintigraphy at every other control

Grade 3
- Close follow-up with ultrasound plus scintigraphy
- Antibacterial prophylaxis could be necessary
- Renal scintigraphy at every other control as far as renal function > 35%
- Surgical (pyelography) indications if significant increase* in hydronephrosis, decrease in renal function

Grade 4
- Early intervention, after short period of follow-up, is safe for prevention of renal function. Delay in prompt treatment may cause irreversible renal deterioration

* Control if the echography is comparable (i.e. empty or full bladder)
Modified from [73].

Differential Diagnosis

The less common mid ureteral tricture accounts for 4% of prenatal hydronephrosis [93].

126.3.7.3 Vesicoureteric Junction Obstruction

Causes of pediatric obstructive hydroureteronephrosis may include primary or congenital vesicoureteric junction obstruction (VUJO) and a secondary form of VUJO (i.e., ureterocele, retroperitoneal fibrosis, calculus).

Diagnosis

An US screening during pregnancy enables the physician to make an early diagnosis of primary VUJO, which involves 4% of the total fetal hydronephrosis [94]. Prenatal diagnosis of VUJO requires postnatal US confirmation and follow-up. Most series have reported an operation rate, predominantly performed because of reduced renal function, in the range 10 to 20%. Prenatally detected VUJO follows an even more benign course than PUJO with a high tendency to spontaneous resolution [95]. However, MRI techniques make it possible to see the anatomical details of the obstruction level from the third trimester of pregnancy to postnatal life [96]. Primary obstruction may become symptomatic late in infancy, especially in patients with normal prenatal US screening [97].

Management

Surgical treatment consists of excision of the distal ureteric segment, tapering of the ureter and reimplantation or insertion of a JJ stent [98].

126.3.8 Vesicoureteral Reflux

VUR is the retrograde flow of urine into the upper urinary tract. The estimated prevalence of VUR affects about 1% of

the general infant population [99]. Mild fetal renal dilatation (fetal renal pelvis 4–10 mm) was associated with VUR ranges between 13 and 30% [99], with an increasing risk in children of parents with VUR. Infants with a family history of VUR, persistent fetal renal dilatation after 30 weeks of gestation should be investigated [100].

Generally, in newborns a reflux is primary. Primary reflux can be associated with dysplastic kidneys, especially in boys [101]. There is growing evidence that congenital renal dysplasia rather than acquired infective scarring is the most important determinant of reflux nephropathy in males with high-grade VUR, who are the infants most commonly identified by prenatal US [65]. The mean overall relative risk of kidney damage is higher in renal units with VUR, particularly in those carrying high-grade VUR, than in not-refluxing kidney [97].

Some cases of VUR are associated with duplicated renal tracts: the lower ureter may be refluxing since it inserts laterally into the bladder. The upper moiety might be associated with an obstructed ureter that inserts into the bladder in a more distal position than normal, or inserts ectopically, for example into the urethra. Finally, some cases of VUR are secondary to other conditions, such as fetal bladder outflow obstruction from PUVs [102].

126.3.8.1 Postnatal Management

Three methods are currently used to identify VUR in children: X-ray voiding cystourethrography (VCUG), radionuclide voiding cystography, and echo-enhanced voiding urosonography [103]. Diagnosis of VUR is traditional performed with serial VCUG scans. In young patients, US showed higher diagnostic accuracy than radionuclide cystography in assessing the grade of VUR [104].

Treatment goals include the prevention of pyelonephritis, reflux nephropathy, and other complications of reflux. Treatment alternatives include antibiotic prophylaxis and surgical correction (open, injection therapy, or laparoscopic) performed after the neonatal period [105], although the additional benefit of surgery over antibiotics alone is small at best [106]. The treatment of choice, with antibiotic prophylaxis and/or with surgery, needs to be discussed on an individual basis.

126.3.8.2 Prognosis

Surgery procedure (ureteral reimplantation or endoscopic correction) leads to similar renal outcomes as medical or supportive treatment. Generally, a congenitally damaged kidney does not ameliorate after each treatment. However, prenatal detection of VUR does not seem to modify the outcome of the kidney significantly [29]. Despite treatment of affected children for the past 40 years, the incidence of end-stage renal disease secondary to VUR has not decreased [107]. Thus, the association of VUR and renal failure may be caused by a genetic defect affecting both the formation of the kidney and the urinary tract [20].

126.3.9 Ureteral Duplications and Ectopia

Ureteral duplications arise from one of two embryological anomalies: a single ureteral bud branching into two before reaching the metanephric mesoderm or two ureteral buds arising from the mesonephric duct and each individually reaching the metanephros to induce formation of two renal moieties.

Ectopia occurs when the ureteral bud arises from an abnormal position on the mesonephric duct. The site of origin of the ureteral bud determines whether the ectopic ureter opens into or outside the bladder. The latter occurs in females and the ectopic orifice is frequently associated with the genital tract.

Ureteroceles represent cystic dilatation of the submucosal part of the ureter. Ureteroceles may be associated with single systems but are more commonly seen in duplex systems [1].

126.3.9.1 Clinical Presentation

Prenatal US scans are capable of identifying most urinary tract abnormalities including duplications, ectopic ureters and ureteroceles. Postnatally many infants may be completely asymptomatic and the diagnosis may be missed in the perinatal period if routine US scans are not available. They may also be associated with obstruction of the upper tracts causing hydronephrosis.

The common postnatal presentation includes UTIs, bladder outlet obstruction and incontinence. Ureteroceles may prolapse through the urethra and present as a mass causing obstruction. Such cases may present with abdominal distension and palpable masses due to the dilated ureter and pelvicalyceal system. Ectopic ureters in males always insert proximal to the sphincter.

126.3.9.2 Diagnosis

US scan is usually the first line of investigation and will show up hydronephrosis, renal dysplasia, dilated ureters, ureteroceles and indicate duplex systems. VCUG will demonstrate the presence of ureteroceles. Moreover, it will show reflux and dilated ureter or part of the duplex system. Radionuclide scan (DMSA) is the most useful for detecting duplex systems and providing an assessment of renal function. Endoscopy provides direct visualisation of ureteroceles and some ectopic ureters. Contrast studies in retrograde fashion improve the delineation of the anatomy.

126.3.9.3 Management

Ectopic ureters

If renal function is adequate, the procedure of choice is reimplantation of the ureter with single system ureters. In duplex systems, either reimplantation of both ureters or anastomosis to the lower moiety ureter as an uretero-ureterostomy may be required. Nephroureterectomy is advisable with absent or poor renal function in duplex system. Removal of the ureteric remnant can be mandatory when there is reflux into the ectopic ureter [108].

Ureterocele

The initial management in the neonate is cystoscopic incision to provide drainage. Subsequently, the renal morphology and function can be assessed with follow-up US and DMSA scans. If these remain satisfactory and no UTIs occur, the patients do not require further surgical intervention [109]. Incomplete excision can leave a cuff of ureterocele at the bladder outlet that acts as a valve obstruction when the patient voids. Finally, complications are infections, obstruction and non-function of the renal moiety requiring treatment by nephrectomy with complete excision of the ureterocele.

Ureteral Duplication

The most common congenital abnormality of the urinary tract is ureteric duplication. Many children with renal tract duplications remain asymptomatic and do not require surgical intervention. Therefore, ureteric duplication is frequently associated with other urinary tract anomalies. Duplex systems are prone to UTIs because of VUR or obstruction. Hydronephrosis in complete duplicated systems usually affects the upper pole, and is mostly secondary to obstruction at the lower end of its ureter by an ureterocele or due to an ectopic ureteric insertion. Hydronephrosis of the lower segment is most often a result of VUR. Obstruction at the level of the PUJ in duplex kidneys is rare [110].

When symptoms occur in duplex system, surgical management is required [111]. Duplications associated with poorly functioning renal moiety and complicated by UTIs should be treated by heminephroureterectomy. Duplications with preserved renal function but complicated by obstruction or infection should be treated by reimplantation of the ureter/ureters. All of the surgical procedures can be done laparoscopically with equally good results compared to open surgery. The ultimate goal of surgical intervention remains the elimination of complications associated with urinary tract abnormalities in the perinatal period and the preservation of renal function.

126.3.10 Anomalies of the Bladder

Bladder exstrophy is a complex anomaly involving the urinary, genital, and intestinal tracts and the musculoskeletal system. The severity ranges from simple epispadias to complete exstrophy of the cloaca involving exposure of the entire hindgut and the bladder. The diagnosis is made typically at the newborn examination or on fetal US that is performed by an experienced observer [112]. Exstrophy of the urinary bladder occurs about once in every 30,000–50,000 births, with male preponderance [113].

126.3.10.1 Clinical Manifestations

Anomalies of the bladder are hypothesized to result when the mesoderm fails to invade the cephalad extension of the cloacal membrane; the extent of this failure determines the degree of the anomaly. In classic bladder exstrophy, the bladder protrudes from the abdominal wall and its mucosa is exposed. The umbilicus is displaced downward, the pubic rami are widely separated in the midline, and the rectus muscles are separated. In males, there is complete epispadias with dorsal chordee, and the overall penile length is approximately half that of unaffected boys [114]. Undescended testes and inguinal hernias are common. Females also have epispadias, with separation of the two halves of the clitoris and wide separation of the labia. The anus is displaced anteriorly in both sexes, and there may be rectal prolapse. The pubic rami are widely separated.

Children with more complex cases of cloacal exstrophy associating with omphalocele, exstrophy of the cloaca and imperforate anus had a prevalence of 1:200,000 live births. The real incidence is unknown since many cases are incorrectly diagnosed prenatally or these pregnancies are prematurely terminated [115]. However, other severe abnormalities of the colon and the rectum are present such as short bowel syndrome [116], upper urinary tract anomaly and spina bifida. Current reconstructive techniques result in a satisfactory outcome in most patients.

Epispadias is in the spectrum of exstrophy anomalies, affecting approximately 1 in 117,000 boys and 1 in 480,000 girls. Epispadias, classic bladder exstrophy and cloacal exstrophy are causally related, representing a spectrum of the same developmental defect, with a small risk of recurrence within families [117]. In boys, the diagnosis is obvious because the prepuce is distributed primarily on the ventral aspect of the penile shaft and the urethral meatus is on the dorsum of the penis. In girls, the clitoris is bifid and the urethra is split dorsally.

126.3.10.2 Treatment and Prognosis

Initially, the bladder should be covered with plastic wrap to keep the bladder mucosa moist. These individuals are prone to

latex allergy, so latex precautions should be practiced in their care. Early bladder closure with reconstructive surgery can be applied to almost all neonates with classic bladder exstrophy. Conventional therapy has included a series of staged reconstructive procedures, but a single-stage complete reconstruction in the neonatal infant has gained popularity [118]. Treatment should be deferred in selected situations when surgical therapy would be excessively risky or complex, such as in a premature baby or when it would have to be performed by inexperienced surgeons. Postoperatively, the infant's upper urinary tract is monitored closely for the possible development of hydronephrosis and infection. Most infants with bladder exstrophy have VUR and should receive antibiotic prophylaxis.

Children who undergo reconstructive surgery as newborns have a greater chance of obtaining a normally functioning bladder.

126.3.11 Anomalies of the Penis

Hypospadias is one of the most frequent genital malformations in the male newborn and results from an abnormal penile and urethral development; estimates of its prevalence range from 3–8 cases per 1000 male births [119]. Hypospadias referring to an incomplete development of the prepuce, called a dorsal hood, in which the foreskin is on the sides and dorsal aspect of the penile shaft and absent ventrally. Hypospadias shows a genetic pattern especially in familial and syndromic forms and in forms due to abnormal genital development or associated with a defect of the androgens pathway [120].

126.3.11.1 Clinical Manifestations

Hypospadias is classified according to the position of the urethral meatus after taking into account whether chordee is present. The deformity is described as glanular (on the glans penis), coronal, subcoronal, midpenile, penoscrotal, scrotal, or perineal. In the most severe cases, the scrotum is bifid and sometimes extends to the dorsal base of the penis [121]. Approximately 10% of boys with hypospadias have an undescended testis; inguinal hernias also are common [122]. In the newborn, the differential diagnosis of proximal hypospadias associated with an undescended testis should include forms of ambiguous genitalia, particularly female virilization (congenital adrenal hyperplasia) and mixed gonadal dysgenesis. A karyotype should be obtained in patients with midpenile or proximal hypospadias and cryptorchidism.

126.3.11.2 Treatment

Management begins in the newborn period. This malformation is usually corrected surgically when the infant is between 6 and 24 months. Whereas hypospadias repair is recommended for boys with midpenile and proximal hypospadias, some boys with distal hypospadias will have no functional abnormality and do not need any surgical correction.

126.4 Differential Diagnosis

Although histology provides the purest way of classifying renal tract malformations, renal biopsies are rarely performed in suspected cases of dysplastic or hypoplastic kidneys. Consequently, in clinical practice, most diagnoses of renal tract malformations are made on the basis of radiological investigations [123].

126.4.1 Fetal Radiology

Prenatal diagnosis can identify either specific pathologies (MDK, duplex collecting system, renal agenesis, PKD) or non-specific pathologies (hydronephrosis, ureteral dilatation, bladder enlargement and oligohydramnios, hyperechogenicity, enlarged kidneys, hypoplastic kidneys). US screening for fetal anomalies is becoming routine and abnormally shaped bladders from midway through gestation [39]. The finding of a renal tract malformation during antenatal screening leads to careful evaluation of the rest of the fetus. In some cases, other organ systems are found to be malformed, which can indicate the presence of a particular syndrome [124].

MRI contribution to the analysis of urinary tract abnormalities is far less well-developed, although this method has proven useful in small series [75, 96, 125]. With regard to nephropathy, the potential contribution of MRI is not currently well-defined and US examination is still the major imaging tool, despite its usefulness in cases with inconclusive sonographic findings (maternal obesity and/or oligohydramnios).

126.4.1.1 Fetal Renal Pelvis Dilatation

Fetal renal pelvis dilatation is a frequent abnormality that has been observed in 4.5% of pregnancies [126]. The fetal kidneys can be easily visualized on US scans at 12 weeks. After 16 weeks, fetal urine production becomes the primary source of amniotic fluid [72]. US provides the opportunity to diagnose upper tract obstruction based on measurements of the renal pelvic APD. Measurements of 3 mm or less are considered normal at any gestational age. It is generally accepted that a fetal renal pelvic diameter greater than 10 mm at 26 weeks gestation is indicative of significant dilatation. In particular, fetal renal dilatation after 23 weeks can be used to predict obstructive uropathy, especially if the renal pelvic diameter continues to increase throughout pregnancy [127]. The

threshold for the diagnosis of abnormal fetal renal pelvis dilatation is significantly higher among pediatric urologists than nephrologists.

Postnatal renal pelvis dilatation is considered abnormal if the APD was 11 ± 1.9 mm by the pediatric urologists and 9 ± 2.9 mm by the pediatric nephrologists [93]. The third-trimester threshold value for renal pelvis APD of 7 mm is beyond any doubt the best prenatal criterion both for the screening of urinary tract dilatation and for the selection of patients needing postnatal investigation. In cases where only a second-trimester fetal examination is performed, a 4 mm threshold value for pelvis APD should be used as a warning sign because this finding may reveal a significant urologic abnormality in 12% of cases [44]. Hydronephrosis has an association with aneuploidy and is a component of several well-described syndromes if associated with other organ system (VACTERL association). The risk for trisomy 21 is approximately 1.5 times the patient's background risk [128].

VUR is diagnosed in 9–15% of infants with prenatally detected hydronephrosis and persistent postnatal hydronephrosis with VCUG performed during the first week of life. Prenatally detected VUR is more frequent in boys and is associated with poorly functioning kidneys in 15–30% of infants without a previous history of UTI [129].

126.4.1.2 Renal Agenesis

Diagnosis of renal agenesis can be complicated when the fetal adrenal gland occupies the empty renal bed, since the adrenal gland can mimic a kidney in US scans.

126.4.1.3 Dysplastic Kidney

MDK and severely hydronephrotic kidneys appear as collections of hypoechogenic spaces. These spaces are noncommunicating with the renal pelvis in multicystic disease and communicate in hydronephrotic ones. Moreover, increased echogenicity in variably enlarged fetal or neonatal kidneys is a nonspecific finding associated with polycystic and cystic dysplastic kidneys [130].

126.4.1.4 Posterior Urethral Valves

PUVs are characterized by variably enlarged, thick-walled urinary bladders and a dilated anterior urethra, both of which findings are highly variable and not specific for the condition [131]. Other entities, such as urethral atresia and prune belly syndrome, can mimic the radiological appearance of PUVs. Prune belly syndrome involves severe dilatation of the lower renal tract in the absence of overt anatomical obstruction, in association with deficiency of the abdominal wall musculature and cryptorchidism [132].

126.4.1.5 Polycystic Kidney Disease

In PKD, the US prenatal screening report unspecific hyperechogenic kidneys. Therefore, fetal MRI is not disadvantaged by a lack of amniotic fluid and, in addition to renal analysis, can be used to look for pulmonary hypoplasia or associated brain malformations. Fetal MRI images with high resolution allow the visualization of cysts in cases of ARPKD [96, 133]. In particular, fetal MRI appearance of ARPKD has been described as increased renal signal intensity on T2-weighted images [134], whereas low renal signal intensity is observed on T1-weighted images because of high water content in the renal parenchyma, consistent with tiny renal cysts. RARE-MR urography can be used to show directly the microcystic dilatation of collecting ducts [135].

126.4.2 Postnatal Radiology

Generally, postnatal neonatal radiology includes US, CT and MRI. Considering the immaturity of nephrons in newborns, the usual recommendation is that renography would be delayed until the age of 4 weeks [43].

Scintigraphy helps establish correct and accurate diagnosis in most congenital renal anomalies, indicates the current functional state of the individual kidneys, provides prognostic information, and can be used for decisions about intervention [136].

126.4.2.1 Hydronephrosis

The objectives of postnatal evaluation are to confirm hydronephrosis and then to determine its cause and to assess renal function. Neonatal urine generation can take a few days to be fully established. Hydronephrosis can be missed in cases of urinary flow obstruction if US scanning is performed too soon. Remarkably, the first postnatal US study is usually performed during the first week after birth, depending on the severity of the prenatal findings, but not during the first 72 h because of reduced urine output after delivery. Hydronephrosis does not generally represent a surgical emergency in the newborn. Most infants probably do not need surgery. The decision should be taken only on the basis of at least two evaluations demonstrating either a progressive increase in dilatation or a progressive reduction in glomerular filtrate. Normal appearing urinary tracts on two successive neonatal US rarely coexist with abnormal findings at VCUG [137]. Unilateral hydronephrosis is generally considered a benign condition that rarely leads to renal failure. Follow-up would appear to be mandatory in all patients, even over lengthy periods. Prenatal renal pelvic dilatation correlates with obstruction, and 15 mm maximum dilatation appears to be the most accurate predictor of postnatal

obstruction [138]. Bilateral hydronephrosis requires prompt evaluation by US and VCUG, especially in boys, in order not to miss PUVs [71, 139].

126.4.2.2 Dysplastic Kidneys

MDKs often regress at US in the first few postnatal years [140]. Therefore, it is infrequent that kidney remnant remain below the limit of radiological detection. Moreover, involution of noncystic dysplastic kidneys can also occur [62, 141]. In a child with a CSFK, it is difficult to distinguish either unilateral renal agenesis or aplasia from a regressed dysplastic kidney unless prenatal data are available. Large cysts replacing kidney parenchyma and lack of central pelvis or tiny remnant kidney characterize MDK. Cystic dysplastic kidneys have echobright kidney, echobright kidney with cysts and poor corticomedullary differentiation [142]. Renal agenesis and MDK commonly affect only one renal tract, although the contralateral tracts have an increased incidence of PUJO and primary VUR [143, 144]. ARPKD have bright kidney, echobright, large kidney with small cysts. ADPKD have large, bright kidney, sometimes with cysts that increase in size and number with age.

126.4.2.3 Renal Agenesis and Hypoplasia

Renal agenesis or aplasia is characterized by absent kidney at fetal US (adrenal gland might be mistaken for kidney). In CSFK, the kidney might be larger than normal, whereas it is hypoplasic when small kidney at US [145]. Some excretory functional compensation occurs postnatally in humans with bilateral dysplastic or hypoplastic kidneys. Distinguishing between dysplastic remnants and aplastic kidneys can be clinically relevant, because dysplastic remnants can sometimes drive hypertension [146]. Not all small kidneys, detectable by US and DMSA scintigraphy, may be caused by developmental malformations. Moreover, neonatal renal vein thrombosis [147] or severe renal artery stenosis can all damage a normal kidney, resulting in a small organ [148].

126.4.2.4 Vesicoureteral Reflux

VUR is the main risk factor for nephropathy [149]. The histological finding of dysplastic changes was reported in some poorly functioning kidneys attached to ureters with primary VUR [101]. Some of the babies with VUR, especially the boys, showed parenchymal defects, without history of UTIs [150]. In these subjects, the scarring associated with primary VUR were related as congenital kidney malformations. On the other hand, in girls, renal parenchymal changes associated with primary VUR were more commonly associated with a history of UTIs [151].

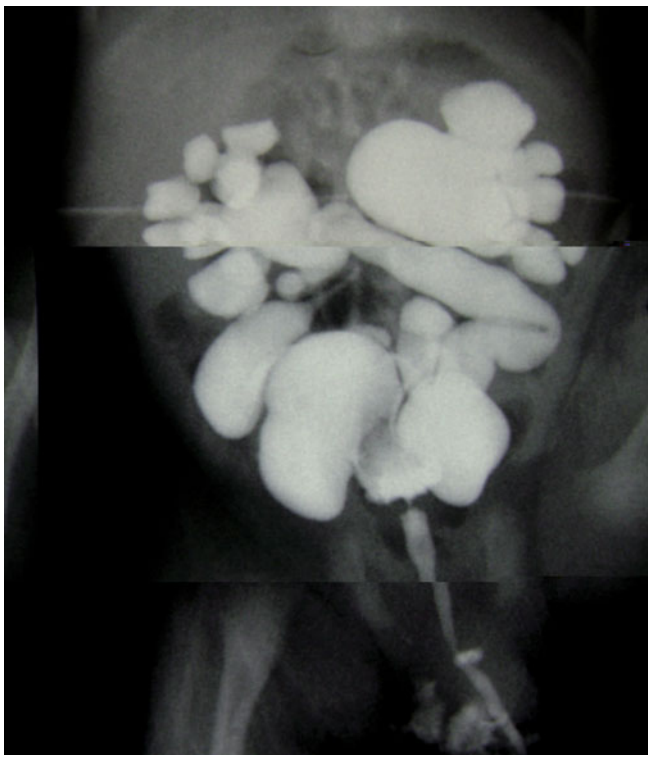

Fig. 126.3 Voiding cystoureterogram in a baby with posterior urethral valves

Scintigraphy with DMSA scan is currently used to detect nephropathy from VUR since concentrates only in functioning tubules. The DMSA scintigraphy may detect focal parenchymal defects that in most cases occur transiently after acute pyelonephritis but that resolve over 3–12 months [152].

126.4.2.5 Posterior Urethral Valves

PUVs are diagnosed by VCUG or cystoscopy revealing an obstruction at the urethral level in males (Fig. 126.3). VCUG can reveal the presence, grade and position of the VUR. In addition, dynamic radioisotope renography MAG3 can provide information about the flow of urine. Drainage from a very-dilated upper tract might be slower than normal even if there is no anatomical obstruction.

126.5 Prognosis

126.5.1 Antenatal Hydronephrosis

Children with any degree of antenatal hydronephrosis are at greater risk of postnatal pathology as compared with the normal population. Favorable prognosis in children with

antenatal hydronephrosis is related with monolateral pathology, such as such as renal agenesis or MDK, late obstruction and renal pelvis dilation. These forms are associated with normal amniotic fluid and normal lung development and the risk of renal failure in childhood is minimal [47, 143]. Although prenatal hydronephrosis has good prognosis, it raises the risk of hospitalization for pyelonephritis in infancy [153]. There was a significant increase in risk of pathology per increasing degree of hydronephrosis. The risk of any postnatal pathology per degree of antenatal hydronephrosis was 11.9% for mild, 45.1% for moderate, and 88.3% for severe [154]. Spontaneous resolution of idiopathic prenatal hydronephrosis occurs in most cases when the renal pelvic APD is less than 12 mm, but is less frequent when dilatation is greater than 12 mm [155].

126.5.2 Renal Agenesis and Dysplasia

Non-favorable renal prognosis is related to bilateral pathology, such as bilateral agenesis, bilateral MDK, early urethral obstruction (PUVs). Generally, these forms are associated with oligohydramnios and abnormal lung development. The renal functional outcomes in children with bilateral renal pathology are worse than those in children with uncomplicated unilateral disease [156], and decreased glomerular filtration rate (GFR) at presentation predicts progression to renal failure during childhood [142]. On follow-up, unobstructed and obstructed dysplastic or hypoplastic kidneys together account for about 40% of all children on renal replacement therapy. Dysplastic or hypoplastic kidneys are 10 times more common than PKD in children with renal replacement therapy [3].

126.5.3 Vesicoureteral Reflux

The risk of VUR was similar for all degrees of antenatal hydronephrosis [154]. In most infants with VUR, the reflux is of low grade and resolves rapidly. Reflux resolved spontaneously in 56% of cases at the 12-month follow-up and 72% at the 24-month follow-up. In those children with high-grade VUR, spontaneous resolution is rare at age 2 years. High-grade VUR had complete resolution of reflux in 9% at 12 months and 18% at 24 months [157]. The outcome of severely refluxing renal units was similar after medical treatment or surgical correction. Finally, prenatal and postnatal diagnosis of hydronephrosis from VUR did not seem to modify the renal outcome at follow-up [29].

126.5.4 Pyeloureteral Junction Obstruction

A prospective study on unilateral congenital PUJO showed that in kidneys with good function, followed conservatively, about a quarter underwent pyeloplasty because of an observed decrease in function [158]. Obstructed kidneys may undergo to stabilization or a relative reduction of the scintigraphic renal function than the unaffected contralateral kidney [159]. In this latter case, failure of the pyeloplasty could be due to underlying concomitant congenital renal dysplasia.

126.5.5 Posterior Urethral Valves

For several decades in newborns with PUVs it has been technically feasible to decompress fetal bladders affected by outflow obstruction by use of vesico–amniotic shunts. Unfortunately, no clear improvement in renal outcomes with such interventions has been shown, but only weak evidence that perinatal survival increased [77]. Although fetal intervention has the capacity to reduce neonatal mortality from pulmonary hypoplasia, increased survival of infants with severe renal dysplasia are destined to progress rapidly into ESRF.

126.6 Overview of Treatment Options

The most severe types of malformations, such as bilateral renal agenesis or dysplasia, although rare, lead to renal failure. With advances in dialysis and transplantation for young children, it is now possible to prevent the early death of at least some individuals with severe malformations. Now intervention could be either prenatal or postnatal for some disease. Treatment options for specific diseases are discussed in the related sections.

126.6.1 Fetal Intervention

Therapeutic termination of pregnancy is often undertaken if a fetus has severe renal tract malformations [39]. The main factor that leads to the decision to terminate is poor renal function and/or severe bladder outflow obstruction, which is manifested by reduced amniotic fluid, sometimes leading to impaired lung growth (oligohydramnios sequence or Potter's syndrome). However, some fetuses are found to have anomalies in other organ systems (e.g., brain, heart or gut), which are sometimes accompanied by gross chromosomal aberrations, such as trisomies [160]. With advances in dialysis for young babies and renal transplantation for young children [161], termination might not always be necessary [162].

The long-term outcomes for children born with PUVs remains poor, with some dying perinatally for lung hypoplasia and respiratory failure, and a significant subset of survivors going into severe renal failure within the next two decades of life [77, 163]. Even if infants with PUVs undergo bladder decompression, the risk of developing ESRF rises

with increasing age [164]. The long-term protection of kidney function from PUVs with prenatal surgical decompression aimed to slow the progression of renal failure needs to be established as well [2].

Therefore, prenatal diagnosis may have made an important, although numerically small, contribution to reducing the risk of renal failure in significant obstruction or high-grade reflux in a solitary functioning kidney.

126.6.2 Postnatal Therapies

The association between chronic pyelonephritis and VUR in childhood suggested an active treatment of individuals born with primary VUR to minimize kidney damage. The treatment options for specific disease are discussed in each section. The postnatal therapeutic options include surgery for obstructed kidneys and severely refluxing kidneys that are rarely performed before the first month of life and antimicrobial prophylaxis to treat and prevent infective complications.

126.6.3 Non Obstructed Hydronephrosis

Early correction of VUR in neonatal period is not performed since most of VUR resolve spontaneously. Few data support the primacy of either long-term antibiotic therapy or antireflux surgery for renoprotection [105, 165]. The era of active therapy has not reduced the incidence of end stage renal disease (ESRD) that occurs as a result of reflux nephropathy, which accords with the concept that reflux nephropathy is often caused by dysplasia or hypoplasia rather than by pyelonephritic damage [106]. Finally, after postnatal ages the initiation of therapies such as with prophylactic antibiotics, to prevent UTIs [166] or angiotensin blockade for reflux nephropathy [167], may be indicated in selected cases to improve the long-term renal outcomes.

126.6.4 Urinary Tract Obstruction

Rapid decline of the renal function related to severe obstruction, such as in newborns with solitary kidney, needs to be discussed for early surgical intervention with an expert pediatric urologist, as well as for early postnatal ablation of the urethral valves in newborns since early ablation and bladder management gave a better outcome [168]. Long-term results of prenatally detected PUVs confirm that early valve ablation can be considered as the primary treatment in the majority of patients, without the need for preoperative drainage or diversion. Gestational age at diagnosis and volume of amniotic fluid are significant predictors of postnatal renal outcome [83]. Moreover, in low birth weight neonates, primary valve ablation by a visually guided Fogarty catheter gave effective disruption of the valvular obstructive mechanism [169].

126.7 Laboratory Management of Kidney Diseases

Laboratory management of renal function is usually performed by urinalysis as well as by determining blood urea nitrogen (BUN) and serum creatinine for the estimation of the glomerular filtration rate (GFR). This traditional strategy, which has existed for more than one hundred years, seems no longer to meet the demands to detect and exclude renal lesions in early and treatable states, especially in the neonatal age [170]. The most important factor appears to be the renal functional reserve that masks renal degeneration, as assessed by GFR, BUN, and creatinine, up to the point where over 75% of the functioning nephrons have been lost. It should be stressed that these factors measure incipient renal failure and in most cases, the finding of normal results does not mean the absence of renal dysfunction. In addition, tubular proteinuria, which reflects tubulotoxic and tubulointerstitial diseases, is likewise not detected by the protein test strip [171]. Therefore, there is a growing demand for a clinically convenient and reliable markers of renal function.

126.7.1 Biomarkers in Pediatric and Neonatal Nephrology

The widespread availability of enabling technologies such as functional genomics and proteomics has accelerated the rate of novel biomarker discovery and therapeutic targets for kidney diseases [172]. The advent of the microarray, or cDNA chip, allows investigators to search through thousands of genes simultaneously, making the process very efficient. Such gene expression profiling studies have identified several genes whose protein products have emerged as chronic kidney disease (CKD) and acute kidney injury (AKI) biomarkers. However, microarray-based methods cannot be used for the direct analysis of biological fluids, and usually require downstream confirmation by proteomic techniques prior to clinical use. Proteomics is the study of both the structure and function of proteins by a variety of methods, such as gel electrophoresis, immunoblotting, mass spectrometry, and enzymatic or metabolic assays. Each method is used to determine different types of information and has its own set of strengths and limitations. Advancing technologies have radically improved the speed and precision of identifying and measuring proteins in biological fluids, and proteomic approaches are also beginning to yield novel and non-invasive biomarkers for assessing kidney damage. Urinary proteins include soluble

Table 126.4 Sources of urinary proteins

Sources of urinary proteins	Comments
1. Soluble proteins	
a. Glomerular filtration of plasma proteins	– Normally present (< 150 mg/day) – Defects in glomerular filter increase high molecular weight protein (e.g., albumin) excretion – Defects in proximal tubule reabsorption or abnormal production of low molecular weight plasma proteins increase low molecular weight protein (e.g., 2-microglobulin, immunoglobulin light chains, retinol-binding protein, and amino acids) excretion
b. Epithelial cell secretion of soluble proteins	Via exocytosis (e.g., epidermal growth factor) or glycosylphosphatidylinositol-anchored protein detachment (e.g., Tamm-Horsfall protein)
2. Solid phase components	
a. Epithelial cells	
– Whole cell shedding	Increased cell number compatible with several diseases including acute tubular necrosis (e.g., renal tubule cell shedding) and glomerular diseases (e.g., podocyte shedding)
– Plasma membrane and intracellular component shedding	Could be due to nonspecific, nephrotoxic, or apoptotic processes
– Exosome secretion	Normal process
b. Other cells	In certain diseases, red blood cells, white blood cells, or tumor cells (e.g., bladder cancer and lymphoma) can be present in urin

Modified from [244].

proteins and protein components of solid phase elements of urine (Table 126.4). Solid phase elements consist of sediments that can be precipitated at low centrifugation speeds and exosomes that are of very low density and sediment only with ultracentrifugation. Prefractionation of these components can be useful as a means of enriching for markers of particular types of disease. A study of urine collected from normal human adult subjects indicated that, of the total urinary protein excreted, ~48% was container in sediments, 49% was soluble, and the remaining 3% was in exosomes [173].

Some of the soluble proteins in urine originate as membrane-bound proteins that are proteolytically cleaved from their membrane attachments. One of these is Tamm-Horsfall protein (uromodulin), an abundant soluble urinary protein that is secreted by the thick ascending limb of Henle loop, a nephron segment downstream from the proximal tubule [174]. It originates as a glycosylphosphatidylinositol-linked protein present in the apical plasma membrane that can be cleaved from its cell attachment proteolytically.

126.7.2 Markers for Assessing Glomerular Filtration Rate

Serum creatinine concentration and endogenous creatinine clearance have been used for such a long time to assess glomerular filtration rate (GFR); nevertheless, serum creatinine has a limited diagnostic value, because this marker shows no response until the GFR is reduced by approximately 40% [175]. Creatinine clearance is affected by inaccuracies in quantitatively collecting urine and by the renal tubular secretion of creatinine, which would falsely elevate the apparent GFR [176]. Moreover, proteins and substances with a ketone group are known to interfere in the Jaffe (alkaline picrate) reaction for the measurement of creatinine in serum and urine; this prevents the measurement of the true concentration of creatinine [177]. Finally there is a lack of availability of pediatric creatinine serum standards referenced to an isotope dilution mass spectrometry method [178].

Because serum creatinine concentration is influenced by both the production rate and the excretion rate, the nephrology community concluded that results should be interpreted in light of the expected rate of production of creatinine. Thus, equations based on serum creatinine are more accurate and precise than serum creatinine alone for estimating GFR (*e*GFR), as recommended by the clinical practice guidelines for CKD in children [179]. Estimating equations include variables such as sex, age, body size, and ethnicity in addition to serum creatinine, as surrogates for muscle mass. Equations have the advantage of providing an estimate of GFR which empirically combines all of these average effects while allowing for the marked differences in creatinine production between individuals [180]. Clinical laboratories should report an estimate of GFR using a prediction equation, in addition to reporting the serum creatinine measurement [181]. Two of the common formulas used for adults are the Cockcroft–Gault and the Modification of Diet in Renal Disease (MDRD) formulas, but these are not appropriate for use in children and babies [182]. In the newborn and in the early infancy, the most widely used estimate of GFR is the original Schwartz equation [183]; based on serum creatinine (SCr), height, and an empirical age-related constant k, this equation was firstly devised in the mid-1970s. It has been successful because it relates GFR to (patient's height)/(SCr) rather than to 1/(SCr). Although simple to use, the Schwartz formula may give biased and imprecise *e*GFR, unless the values of k are derived from local estimates of mean height,

GFR, and SCr. When locally derived estimates of *k* are used, the bias decreases substantially but the precision of *e*GFR remains poor. In addition, the Schwartz equation assumes that there is no intercept associated with the relationship of GFR versus height/SCr which, if incorrect, could lead to more biased *e*GFR and to incorrect values for the slope, *k*. It remains unknown whether a less biased and more precise estimation of GFR can be achieved using regression to determine the relation between GFR and height/SCr in a local population, rather than substituting variables and computing mean *k* [184]. Finally, pediatricians need to recognize that the formula requires updating when analytically specific methods of measuring serum creatinine, such as enzymatic assays, are used in their institutions [185].

The accuracy of the *e*GFR is important for the early detection of CKD in adults, children, and newborns, as has been recognized in the recent awareness campaigns in the European Union and in the US (www.NKDEP.nih.gov). An early detection of changes in GFR leads to an early appropriate treatment options, such as weight loss, exercise, or blood pressure control, especially with angiotensin-converting enzyme (ACE) inhibitors, slowing or even halting progression of renal injury and/or dysfunction. Because serum creatinine concentration and the *e*GFR are interrelated, the performance of the creatinine analysis affects GFR estimates [186]. The Laboratory Working Group of the National Kidney Disease Education Program (NKDEP), in collaboration with international professional organizations, has developed a plan that enables standardization and improved accuracy (trueness) of serum creatinine measurements in clinical laboratories worldwide. The Working Group developed a report in the US, reviewing the information on the non-specificity of routine creatinine methods and recommended that manufacturers of *in vitro* diagnostics address and reduce the influence of interfering substances in patient samples [187].Two serum pools with creatinine concentrations within the pediatric reference interval were spiked with albumin, IgG, unconjugated bilirubin, adult hemoglobin (Hb A), and fetal hemoglobin (Hb F) to produce one unspiked and five spiked samples per pool. Albumin, IgG, and Fetal Hemoglobin (HbF) interfered with Jaffe creatinine assays, leading to inaccuracies in *e*GFRs that are clinically important, especially in children and neonates. In particular, albumin interference leads to a GFR underestimation ranging from 17 to 27%, while HbF interference to a GFR overestimation ranging from 24 to 60%. Because protein error and HbF interference do not occur with any of the enzymatic methods tested, the authors conclude that enzymatic creatinine methods are preferred for evaluation of kidney function in pediatric cases. The more recent enzymatic creatinine method results in lower determinations compared with the older Jaffe method, even when the latter was improved with a dialysis step and elimination of interfering samples [188].

In 2009, Schwartz proposed a new formula for calculating *e*GFR in children with CKD [189]. The new equation includes serum creatinine, measured by an enzymatic analytical method, cystatin C, and BUN together with an accurate height measurement. Based on a carefully described, prospectively recruited cohort of children with various types of kidney diseases that have compromised function, the results seem to provide a more accurate, noninvasive method of estimating the GFR in the pediatric and neonatal age. This formula yielded 87.7% of *e*GFR within 30% of the GFR assessed by iohexol plasma disappearance and 45.6% within 10%. This new equation permits to improve the adjustment of drug dosing and can be used as a research tool. But it can also be used clinically as a confirmatory screening tool for children with impaired renal function and to determine whether CKD in children is stable or progressing. Unfortunately, it cannot yet be used as a general screening tool, since it has not yet been verified in a cohort of children with normal renal function.

Taking into account the extreme importance of the accuracy in the measurement of serum creatinine when creatinine-based equation are used to estimate GFR [190], the National Disease Education Program (NKDEP) has developed several recommendations for the improvement and development of creatinine assays [187]. These recommendations include optimizing creatinine assays to provide accurate (traceable to MS-IDMS) and precise measurements (imprecision goal of approximately 8% to meet the maximum 10% impact on *e*GFR) particularly at a concentration of 1.00 mg/dL, revising GFR-estimating equations based on more accurate methods, and introducing proficiency testing programs that use commutable serum materials with target values traceable to MS-IDMS procedure [191]. The National Institute for Standards and Technology has developed a reference material at concentrations of approximately 0.80 and 4.00 mg/dL to help manufacturers in the standardization of creatinine assays [192].

126.7.3 Low Molecular Mass Proteins

Over the past 20 years, interest has been shown in the diagnostic power of low molecular mass proteins (low-M_r proteins) in serum and urine for the evaluation of renal function. These proteins are freely filtered through the capillary wall, then reabsorbed and catabolized in the proximal tubular cells. Thus, if the GFR in the kidney is reduced, the serum concentrations of low-M_r proteins increase and may be taken as suitable markers of GFR [193]. On the other hand, if the tubular reabsorption capacity is reduced, or if tubular cells have been damaged by nephrotoxic drugs or agents, the urinary excretion of these proteins increase and may be taken as suitable index of renal tubular impairment and dysfunction [194]. In the normal kidney, the reabsorption of low-M_r proteins by proximal tubular cells is nearly complete so that only trace amounts of these proteins are excreted in urine (approximately 0.1 mg/24 h). As a corollary, low-M_r proteins in urine are very sensitive markers of impaired proximal tubular function. The urinary excretion of low-M_r proteins reflects

the reabsorptive capacity of proximal tubules when the GFR is normal or only slightly abnormal. Herget-Rosenthal have measured urinary excretion of a number of candidate low-M_r-proteins, such as α_1-microglobulin (protein HC), β_2-microglobulin, cystatin C, retinol-binding protein, α-glutathione *S*-transferase, lactate dehydrogenase, and *N*-acetyl-β-(D)-glucosaminidase, early in the course of non-oliguric acute renal failure in humans [195]. In this cohort of patients with established acute renal failure (ARF) from a variety of causes, urinary excretion of α_1-microglobulin and cystatin C were found to be predictive of severe ARF requiring renal replacement therapy, with an area under characteristic curve (AUC) of 0.86 and 0.92 respectively. α_1-microglobulin is a tubular protein that belongs to the lipocalin superfamily, while cystatin C is a cysteine protease inhibitor that is synthesized and released into the blood at a relatively constant rate by all nucleated cells. Both α_1-microglobulin and cystatin C are stable in the urine [196], and can be easily measured by immunonephelometric methods in most standard clinical chemistry laboratories. The predictive role of these urinary proteins in early AKI remains to be determined.

126.7.4 Cystatin C

Cystatin C is a non-glycosylated 13 kDa basic protein that acts as a cysteine proteinase inhibitor and is produced at a relatively constant rate. This constancy is apparently not influenced by the presence of inflammatory conditions, muscle mass, gender, body composition, and age after 12 months [197]. Blood cystatin C level is approximately 1 mg/L in healthy individuals of age > 1y, while in the neonatal age and in the early infancy blood cystatin C is significantly higher [198]. Cystatin C is catabolized and almost completely reabsorbed by renal proximal tubular cells, so that a very small amount is excreted in the urine [199] and cannot be used to calculate a clearance GFR. Inter-individual variation of cystatin C level is significantly less (25%) than that of creatinine (93%) [200]. The upper limit of the population reference interval for cystatin C is seldom more than 3–4 standard deviations (SD) from the mean value of any healthy individual (compared with 13 SD for creatinine). These findings suggest that cystatin C is potentially a better marker than creatinine for detecting impaired renal function. From a number of clinical studies on cystatin C, two key findings are evident. First, the concentration of serum cystatin C correlated better with directly measured values for GFR than did serum creatinine. Second, subtle decrements in GFR are more readily detected by the determination of serum cystatin C than by creatinine concentration [201]. Thus, while cystatin C is not a conventional marker of GFR, reciprocal values of serum cystatin C levels are reasonably well correlated with GFR in adults [202] and in children [203]. Some studies have suggested that the serum concentration of cystatin C might be superior to serum creatinine in distinguishing normal from abnormal GFR [204]. However, because it is metabolized and not excreted, cystatin C cannot be used to measure GFR by standard urinary clearance techniques [205]. Nevertheless, serum cystatin can be used to estimate GFR in milliliters per minute per 1.73 square meters according to the following formula: $\log_{10}(\text{GFR}) = 1.962 + [1.123 * \log_{10}(1/\text{cysC})]$, where cysC is cystatin C [206]. Additional formulae using both cystatin C and creatinine to estimate GFR have recently been reported. Such studies have shown that plasma cystatin C is slightly better than plasma creatinine in diagnosing renal insufficiency but is less sensitive than creatinine clearance or *e*GFR (from $k * \text{L/Pcr}$) [207].

126.7.5 Albumin in Urine and Kidney Disease

The appearance of albumin in the urine has long been recognized as a cardinal feature of kidney disease and more recently has been shown to also be an independent cardiovascular risk factor associated with insulin resistance. Normal human urine contains only very small quantities of albumin, less than 30 mg/d. In a position statement published in 2003, the National Kidney Foundation and the National Institute of Diabetes and Digestive and Kidney Diseases recommend a spot, untimed urine specimen be used for measurement of urinary albumin [208]. Using the ratio of urine albumin to urine creatinine, microalbuminuria is defined as a level of 30–300 mg albumin/g creatinine [209].

Urine albumin has traditionally been screened in the office setting by use of a variety of semiquantitative dipsticks. These tests involve wetting a chemically impregnated test strip with urine. Some commercial dipsticks detect microalbuminuria by albumin binding to a sulfonephthalein dye, which produces a color reaction that is read reflectometrically in a portable urine chemistry analyzer. Comparisons of this dipstick to reference laboratory tests have shown that the sensitivity ranges from 79 to 95.4% and that the specificity ranges from 73 to 81% [210]. Other commercial dipsticks detect albumin by a different chemical method: albumin passes via a wick fleece into a conjugate fleece, where it binds to specific, gold-labeled antibodies, and then flows to a detection pad [211]. A chemical reaction in the detection pad produces a color that is compared visually with color blocks. Comparisons of this test to reference laboratory results have shown that the sensitivity ranges from 93 to 97.1%, and the specificity ranges from 33.3 to 81% [212].

Quantitative measurement of urine albumin are traditionally based on three laboratory methods: immunonephelometry, immunoturbidimetry, and radioimmunoassay (RIA). The performance characteristics of these methods are listed in Table 126.5. Conventional immunoassays suggest that normal humans would only excrete less than 30 mg/d of albumin, whereas the amount of albumin-derived material excreted may range from 1 to 3 g/d [213]. These studies demonstrate that a considerable amount of peptide-derived

Table 126.5 Performances of the most common analytical methods for the measurement of microalbuminuria

Analytical method reproducibility (CV, %)	Interassay	Detection limit
Radioimmunoassay (RIA)	9.2% at 12.2 mg/L 4.8% at 33.0 mg/L	16 µg/L (0.016 mg/L)
Immunonephelometry (Beckman Array Analyser)	4.2% at 12.1 mg/L 5.3% at 45.0 mg/L	2 mg/L
Immunoturbidimetry (Siemens Turbitimer)	4.1% at 10.6 mg/L 2.2% at 43.2 mg/L 4.2% at 77.9 mg/L	6 mg/L
Immunoturbidimetry (Siemens Dimension RxL)	8.5% at 8.0 mg/L 3.4% at 35.0 mg/L	6 mg/L
HPLC	2.4% at 95.8 mg/L	2 mg/L

Modified from [245].

low-molecular-weight fragments is commonly excreted in urine of healthy subjects. The degradation process occurs in tubular epithelial cells, where albumin is endocytosed and trafficked to lysosomes. Once degraded, albumin is exocytosed into the tubular lumen and excreted in urine. The degradation products are exclusively found in urine and they never return in blood stream. This technique is likely to provide new information as a diagnostic tool because changes in the peptide profile, or fingerprint, can reflect renal disease and possibly other diseases, such as cancer.

126.7.6 Novel Markers for Kidney Ischemia, Hypoxia, and Drug-Induced Toxicity

Proteomic approaches have recently yielded additional biomarkers for acute kidney injury (AKI). AKI is a term proposed to reflect the entire spectrum of acute renal failure, a complex disorder that occurs in a wide variety of settings with clinical manifestations ranging from a minimal elevation in serum creatinine to anuric renal failure [214]. In current clinical practice, AKI is typically diagnosed by measuring serum creatinine. Unfortunately, during acute changes in glomerular filtration, serum creatinine does not accurately depict kidney function until steady state equilibrium has been reached, which may require several days.

Conventional urinary biomarkers such as casts and fractional excretion of sodium have been insensitive and non-specific for the early recognition of AKI. Other traditional urinary biomarkers such as filtered high molecular weight proteins and tubular proteins or enzymes have also suffered from lack of specificity and dearth of standardized assays.

Identification of novel AKI biomarkers has been designated as a top priority by the American Society of Nephrology [215]. The concept of developing a new toolbox for earlier diagnosis of disease states is also prominently featured in the NIH Road Map for biomedical research [216]. The application of innovative technologies such as functional genomics and proteomics to human and animal models of kidney disease has uncovered several novel candidates that are emerging as biomarkers and therapeutic targets [217]. Several human models of AKI have consistently demonstrated the presence of apoptotic changes in tubule cells [218]. Importantly, proteomic studies have now identified a multitude of apoptotic pathways, including the intrinsic (Bcl-2 family, cytochrome c, caspase 9), extrinsic (Fas, FADD, caspase 8), and regulatory (p53) factors, that are activated in tubule cells following human AKI [219]. As a consequence of these studies, inhibition of apoptosis has emerged as a promising approach in human AKI [220]. Cell-permeable caspase inhibitors have provided particularly attractive targets for study. In this regard, an orally active small molecule pan-caspase inhibitor (IDN-6556, Pfizer) has been shown to be effective in preventing injury after lung and liver transplantation in animals [221].

126.7.7 Neutrophil Gelatinase-associated Lipocalin (NGAL)

Supavekin also identified neutrophil gelatinase-associated lipocalin (NGAL, also known as *lcn2*) as one of the most upregulated transcripts in the early post-ischemic mouse kidney [222], a finding that has now been confirmed in several other transcriptome profiling studies. Downstream proteomic studies have also revealed NGAL to be one of the earliest and most robustly induced proteins in the kidney after ischemic or nephrotoxic AKI in animal models, and NGAL protein is easily detected in the blood and urine soon after AKI [223]. These findings have spawned a number of translational proteomic studies to evaluate NGAL as a novel biomarker in human AKI.

In a cross-sectional study, subjects in the intensive care unit with established ARF displayed a greater than 10-fold increase in plasma NGAL and more than a 100-fold increase in urine NGAL by Western blotting when compared to normal controls [223]. Both plasma and urine NGAL correlated highly with serum creatinine levels. Kidney biopsies in these patients showed intense accumulation of immuno-reactive NGAL in 50% of the cortical tubules. These results identified NGAL as a widespread and sensitive response to established AKI in humans. Urine NGAL has been shown to predict the severity of AKI and dialysis requirement in a multicenter study of children with diarrhea-associated hemolytic uremic syndrome [224]. Preliminary results also suggest that plasma and urine NGAL measurements represent predictive biomarkers of AKI following contrast administration [225] and in the intensive care setting [226]. In summary, NGAL is emerging as a center-stage player in the AKI field, as a novel predictive biomarker. However, it is acknowledged that the studies published thus far are small, in which NGAL appears to be most sensitive and specific in relatively uncomplicated patient populations with AKI. NGAL measurements may be

influenced by a number of coexisting variables such as pre-existing renal disease [227] and systemic or urinary tract infections [228]. Large multicenter studies to further define the predictive role of plasma and urine NGAL as a member of the putative AKI panel have been initiated; simultaneously, a new chemiluminescent microparticle method, optimized on a fully-automated analytical platform (ARCHITECT, Abbott Diagnostics Inc, Abbott Park, IL, USA) has been developed for the measurement of urine NGAL in the clinical practice and, specifically, in the emergency setting [229]. This new simple method permits to measure urine NGAL in all the clinical laboratories in a time closely comparable with that occurring for measuring creatinine. Thus, both NGAL and cystatin C may represent promising tandem biomarker candidates for inclusion in the blood AKI panel [230].

126.7.8 Kidney Injury Molecule 1 (KIM-1)

Kidney injury molecule 1 (KIM-1) is a type-1 transmembrane protein with glycosylated mucin and IgG-like domains in the ectodomain of the protein and a relatively short intracellular domain that is tyrosine phosphorylated. Downstream proteomic studies have also shown KIM-1 to be one of the most highly induced proteins in the kidney after AKI in animal models, and a proteolytically processed domain of KIM-1 is easily detected in the urine soon after AKI. In a small human cross-sectional study, KIM-1 was found to be markedly induced in proximal tubules in kidney biopsies from patients with established AKI (primarily ischemic), and urinary KIM-1 measured by ELISA distinguished ischemic AKI from prerenal azotemia and chronic renal disease [231]. Patients with AKI induced by contrast did not have increased urinary KIM-1.

Recent preliminary studies have expanded the potential clinical utility of KIM-1 as a predictive AKI biomarker [232]. KIM-1 represents a promising candidate for inclusion in the urinary AKI panel. An advantage of KIM-1 over NGAL is that it appears to be more specific to ischemic or nephrotoxic AKI, and not significantly affected by prerenal azotemia, urinary tract infections, or chronic kidney disease. It is likely that NGAL and KIM-1 will emerge as tandem biomarkers of AKI, with NGAL being most sensitive at the earliest time points and KIM-1 adding significant specificity at slightly later time points.

126.7.9 Additional Protein Markers: CYR61, SSAT, Zf9, TSP-1, IL-18

Gene expression studies have provided several additional clues regarding the AKI proteome, but human data are hitherto lacking. For example, Muramatsu et al have utilized a subtractive hybridization approach to identify *Cyr61* (also known as *CCN1*) as a markedly upregulated gene in the rat kidney very early after ischemic injury [233]. CYR61 protein was induced in the kidney within one hour and detectable in the urine at 3–6 hours after ischemic injury, but not after volume depletion. However, this detection required a complex bioaffinity purification step with heparin-Sepharose beads, and even after such purification, several crossreacting peptides were apparent. A more convenient platform for the evaluation of CYR61 as a urinary biomarker in humans has not been available to date. Zahedi et al described spermidine/spermine N1-acetyltransferase (SSAT), the rate-limiting enzyme in polyamine catabolism, as a novel early biomarker of tubular cell damage after ischemic injury in rats [234]. SSAT protein appears to play a role in the initiation of oxidant-mediated injury to tubules, raising the possibility of inhibition of polyamine catabolism as a future therapeutic approach [235].

Tarabishi et al showed that another maximally induced gene identified very early after ischemic injury in animal models is Zf9, a Kruppel-like transcription factor involved in the regulation of a number of downstream targets [236]. Zf9 protein is markedly upregulated in the postischemic tubule cells, along with its major trans-activating factor, TGF-β1. Gene silencing of Zf9 abrogated TGF-β1 protein expression and mitigated the apoptotic response to ischemic injury *in vitro* [236]. These studies have thus identified a novel pathway that may play a critical role in the early tubule cell death that accompanies ischemic renal injury. Thakar et al have employed transcriptome profiling in rat models to identify thrombospondin 1 (TSP-1), a previously known p53-dependent pro-apoptotic and anti-angiogenic molecule, as another maximally induced gene early after ischemic AKI [237]. The TSP-1 protein product is upregulated in the post-ischemic proximal tubule cells, where it colocalizes with activated caspase-3. TSP-1 null mice were partially protected from ischemic injury, with striking structural preservation of kidney tissue [237]. These results have thus identified yet another previously unknown apoptotic protein that is activated in proximal tubule cells early after ischemic AKI in animals. Transcripts that have been consistently reported to be either upregulated or downregulated in animal models of AKI are listed in Tables 126.4 and 126.5, respectively. While many of them have now been confirmed by downstream proteomic analysis, the majority of these studies remain in the pre-clinical research realm, and convincing data attesting to their utility in human AKI are currently unavailable.

IL-18 is a pro-inflammatory cytokine that is known to be induced and cleaved in the proximal tubule, and subsequently easily detected in the urine following ischemic AKI in animal models [238]. In a cross-sectional study, urine IL-18 levels measured by ELISA were markedly increased in patients with established AKI, but not in subjects with urinary tract infection, chronic kidney disease, nephritic syndrome, or prerenal failure [239]. Urinary IL-18 was significantly upregulated up to 48 hours prior to the increase

in serum creatinine in patients with acute respiratory distress syndrome who develop AKI, with an AUC of 0.73, and represented an independent predictor of mortality in this cohort [240]. Both urinary IL-18 and NGAL were recently shown to represent early, predictive, sequential AKI biomarkers in children undergoing cardiac surgery [241]. In patients who developed AKI 2-3 days after surgery, urinary NGAL was induced within 2 hours and peaked at 6 hours whereas urine IL-18 levels increased around 6 hours and peaked at over 25-fold at 12 hours post surgery (AUC 0.75). Both IL-18 and NGAL were independently associated with duration of AKI among cases.

Urine NGAL and IL-18 have also emerged as predictive biomarkers for delayed graft function following kidney transplantation [242]. In a prospective multicenter study of children and adults, both NGAL and IL-18 in urine samples collected on the day of transplant predicted delayed graft function and dialysis requirement with AUC of 0.9. Thus, IL-18 may also represent a promising candidate for inclusion in the urinary "AKI panel". IL-18 is more specific to ischemic AKI, and not affected by nephrotoxins, chronic kidney disease or urinary tract infections. It is likely that NGAL, IL-18 and KIM-1 will emerge as sequential urinary biomarkers of AKI [243].

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Brain Development and Perinatal Vulnerability to Cerebral Damage

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127.1 Introduction

The recent exponential rise in detailed magnetic resonance (MR) imaging studies has emphasized the concept of gestationally determined regional vulnerability in the brain: the site and nature of the injury sustained being determined by a combination of the characteristics of the insult, the specific tissue and cell vulnerability and the gestation of the infant. The type of insult may also be partly dependent on gestation. However, it is now known that acute perinatal hypoxic ischemic events, previously considered characteristic for the term born neonate presenting with hypoxic-ischemic encephalopathy (HIE), may occur at earlier points in gestation [1, 2]. Nevertheless, such events occur less often in the infant born preterm where lesions develop in similar brain regions and in other areas characteristically more vulnerable in more premature babies (Fig. 127.1). Similarly, white matter (WM) lesions, which are considered the hallmark of injury to the preterm brain because they are characteristic of perinatal injury relating to inflammation, infection or hypoglycemia in the term brain, may also occur in a small percentage of neonates with an encephalopathy (Fig. 127.2) [3].

The regional tissue vulnerability at a given gestation will be determined by the local metabolic requirements in combination with specific cell characteristics, e.g., density of glutamate receptors [4]. Regional tissue vulnerability at different gestational ages has been demonstrated by numerous *in vivo* brain imaging studies, as well as by conventional postmortem examinations. However, animal studies have highlighted the concept of a specific vulnerability by certain cell types, e.g. the subplate neurons [5] and oligodendrocyte precursors [6] are most vulnerable in the preterm brain. In the term brain, excitatory pyramidal projection neurons especially in the deep grey nuclei are at greatest risk during ischemic insults (Fig. 127.1). The vulnerability of a specific cell type at a given gestation relates to characteristics such as the expression of different glutamatergic receptor subtypes that favor calcium

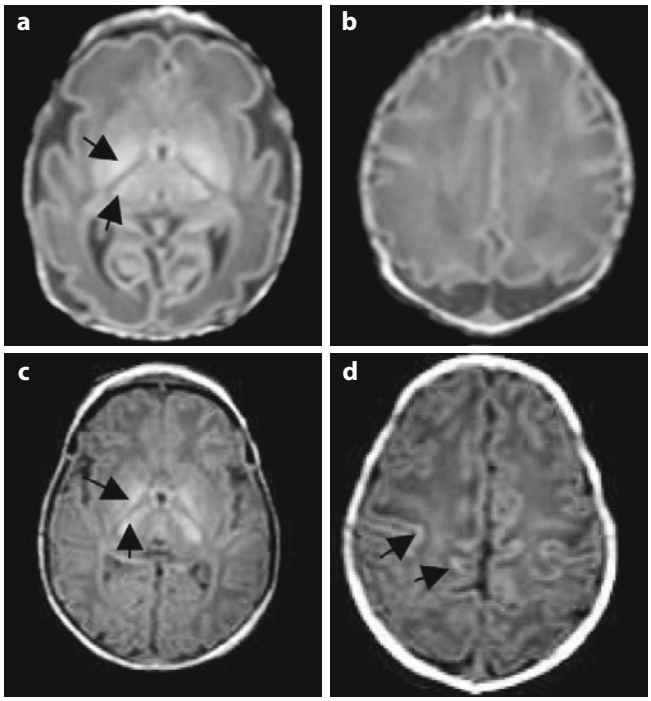

Fig. 127.1 Basal ganglia and thalamic (BGT) injury. BGT lesions are the hallmark of an acute hypoxic-ischemic event regardless of gestational age. **a**, **b** Preterm infant born at 32 weeks gestation by emergency cesarean section (EMCS) for abnormal fetal heart rate. There is abnormal high signal intensity (SI) either side (*arrows*) of the low SI posterior limb of the internal capsule (PLIC) (unmyelinated at this age). There is no obvious abnormality in or adjacent to the developing central sulcus or interhemispheric fissure (**b**). **c**, **d** Term born neonate born by EMCS for fetal distress. There is bilateral abnormal high signal intensity in the globus pallidus and thalamus (*arrows*). There is no myelin in the PLIC, which is abnormal at this age (**c**). Superiorly there is some increased SI in the central sulci and cortex along the interhemispheric fissure (*arrows*). The subcortical white matter adjacent to these regions is abnormally low SI (**d**)

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

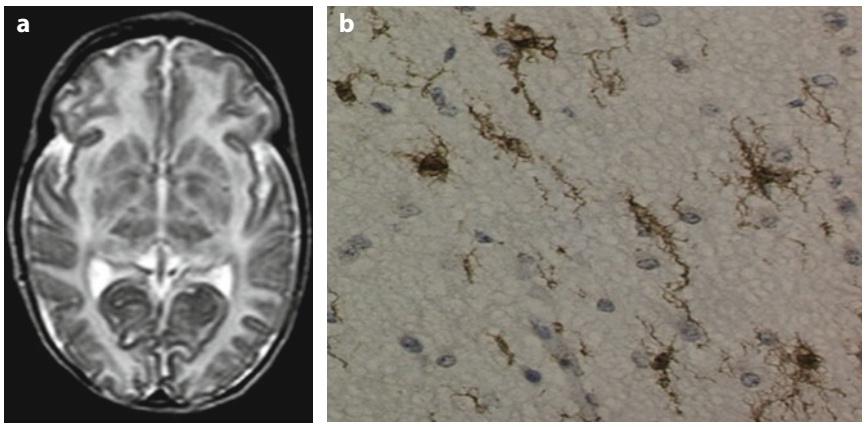

Fig. 127.2 T2 weighted scan (**a**) obtained at 9 days of age in a neonate with severe perinatal basal ganglia and thalamic injury showing diffuse high signal intensity in the white matter. Marked microglial activation (**b**) was seen in the white matter at post mortem ten weeks later

entry and excitability, and endogenous antioxidant mechanisms [7]. In addition, the neonatal neuron is programmed for cell death to allow for essential pruning and optimal connectivity, but this characteristic increases the vulnerability of such cells to injury. However, while research has focussed on WM vulnerability in preterm infants and gray matter vulnerability in term infants, the nature of the insult is also important in dictating lesion site.

In this chapter we will discuss the vulnerability of tissue and cell types in relation to gestational age and examine how these relate to patterns of injury seen on brain MR imaging and the clinical history and presentation of the infant.

127.2 Brain Development

In order to understand the vulnerability of the immature brain to injury, it is essential to consider the stages of brain development from early fetal life until term at around 40 weeks' postmenstrual age (PMA). A full description of brain development is beyond the scope of this chapter so we will concentrate on regions of the preterm brain that are known to be particularly vulnerable; the immature white matter and the germinal matrix. In the more mature term brain we will focus on the deep gray matter and myelinating white matter with different cell types that may contribute to this changing vulnerability.

The first half of gestation is characterized by neuronal proliferation and migration. During the second half, glial cell proliferation and programmed cell death predominate [8–10]. The last trimester of gestation is characterized by developing connectivity. There is axonal and dendritic sprouting and synapse formation. These processes, which involve programmed cell death, continue throughout childhood and into adolescence. Whilst early myelination in central brain structures, such as the brainstem and thalami, is present by 26 weeks, further

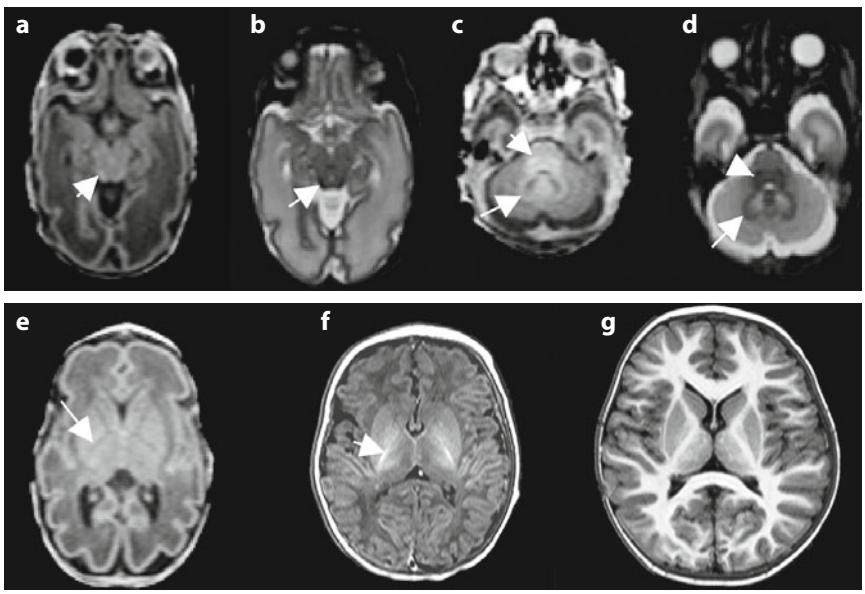

Fig. 127.3 Myelination. Preterm infant born at 26+5 weeks gestation and imaged at 29 weeks (**a**–**e**). Term born neonate (**f**). Two year old child (**g**). Myelination is seen as high signal intensity on T1 weighted images (**a**, **c**, **e**, **f**, **g**) and low signal intensity on T2 weighted images (**b**, **d**). At 29 weeks postmenstrual age myelination is detected in many central brain areas such as in brainstem regions e.g., lateral lemnisci (*arrows*) (**a**, **b**) and the medial lemnisci (*short arrows*) (**c**, **d**) and in the dentate nuclei (*long arrow*) (**c**, **d**). Myelination is easier to detect on T2 weighted images in the preterm brain. Myelination in the PLIC is visible from around 37 weeks postmenstrual age (*arrow*) (**f**). Prior to myelination the PLIC may look low signal intensity on T1 weighted images (*arrow*) (**e**). The majority of brain myelination occurs during the first two years of postnatal life (**g**)

myelination in the posterior limb of the internal capsule is then not detected until the end of gestation [11]. The majority of brain myelination occurs in the first 2 years after birth (Fig. 127.3). There is, however, extensive premyelination occurring within hemispheric white matter during late gestation and the abundance of oligodendrocyte precursors are particularly vulnerable to damage [6]. This pre-myelination is in part responsible for the anisotropy of WM tracts documented in diffusion tensor images of the preterm brain before the appearance of myelination. Measures of anisotropy can therefore be used as an indirect measure of oligodendrocyte injury in the immature brain prior to myelination [11, 12].

Knowledge of the timing of events, such as myelination, allows us to understand the vulnerability of tissue types. *In vivo* methodology to assess such injuring processes provides an insight into the neurological sequelae that may result from lesions sustained at a given gestation.

127.3 Regional Vulnerability

127.3.1 Neuronal Migration

From the fifth week PMA, the neural tube starts to develop and differentiate. Specific proliferation areas can be identified in the ventricular and subventricular zones from where excitatory pyramidal neurons migrate to their final destination, mostly following specialized radial glial fibers. This radial migration process is closely regulated by complex molecular interactions between neurons and glial cells mediated by glycoproteins, membrane lipids and neurotransmitters [13].

More recently it has been appreciated that, in addition to this radial migration, there are two further modes of migration, tangential and multipolar migration [14, 15]. The latter occurs independently of radial glia. It was originally thought that all neuronal migration ceased by 20 weeks PMA, but this referred to radially migrating pyramidal neurons. Gabaminergic inhibitory interneurons generated in the ganglionic eminences migrate in a tangential manner initially and then along the radial glia into the cortex in an inside-out manner. This process continues until past term [8, 16]. Later, the medial and lateral ganglionic eminences appear and give rise to different populations of interneuron; those from the lateral or caudal eminence migrate and occupy superficial layers of the cortex [17]. Thus neuronal migration continues during a period when an infant born preterm may be exposed to a variety of potentially damaging factors. Many recognized disturbances in neuronal migration result from genetic defects [18], giving rise to profound abnormalities of cortical development such as the lissencephalies. Acquired injuries to the immature brain such as ischemia, viral infection [19, 20] or maternal drug abuse [21] may target replicating neuroblasts and disrupt neuronal migration. Acquired injuries result in more focal cortical dysplasias such as polymicrogyria, which is frequently associated with cytomegalovirus infection (Fig. 127.4) [19, 20]. Earlier insults (Fig. 127.4) cause more severe disruption of cortical development, and later insults may result in subtle abnormalities in cortical development that may not be visually obvious but that could be investigated using more advanced imaging techniques such as diffusion tensor imaging.

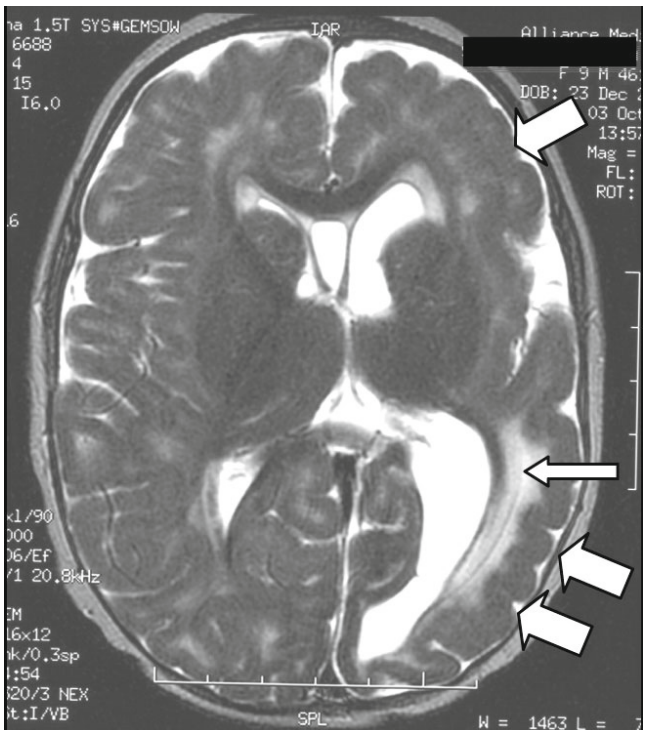

Fig. 127.4 Congenital CMV. Axial T2 scan of a term baby with CMV infection acquired around the 12th week of intrauterine life. The early fetal infection has determined cortical malformations such as polymicrogyria (*white arrows*) more evident in the right hemisphere and diffuse and severe white matter abnormalities (*thin arrow*)

127.3.2 The Cortical Subplate

The cortical subplate is an important anatomical and functional structure, which is present at all phases of intrauterine brain development from early gestation to near term. The first populations of migrating neurons occupy the transient cortical subplate from approximately 7–8 weeks post conception. The subplate is essential for normal cortical development and subplate neurons develop both intracortical and subcortical projections [22]. The subplate zone becomes visible in the human brain at around 14-15 post-conceptional weeks (PCW), the beginning of the second trimester of human gestation. This coincides with the invasion of the subplate region by thalamocortical afferents as well as basal forebrain afferents, leading to rapid expansion of the subplate so that it comprises 35% of the cerebral wall by 16 PCW. From 14 to 25 PCW in the human, a large number neurons are continuously added to the

subplate compartment, which increases in width concurrent with the growth of the cortical plate. The highest density of cells is always found at the border between cortical plate and subplate. At its peak of development, the human subplate zone is four times the width of the cortical plate [23], reaching its maximum thickness in the late second and early third trimester, more or less between 25 and 29 weeks. This is a period of high vulnerability for WM injury. Thereafter it gradually decreases in size and becomes unrecognizable by the sixth month post-term [23].

Early thalamo-cortical afferents remain within the subplate until approximately 28 weeks when they progress into the cortex to form the earliest functional neurocircuits of the neocortex. The subplate layer contains a hydrophilic extracellular matrix that enables visualization of the subplate *in vivo* using MR imaging of the fetal or preterm infant brain. It can be quantified using volumetric scanning or diffusion tensor imaging [24, 25]. On MR imaging the subplate layer is thicker than the cortex until approximately 24 weeks, equivalent to the cortex until about 28 weeks, and then starts to involute, being visible only at the tops of gyri throughout the third trimester (Figs. 127.5, 127.6). A normal term infant usually only displays a few regions of residual subplate on imaging, and, in infants with overt lesions, the subplate may disappear prematurely. In contrast, in preterm infants imaged at term equivalence, the subplate may persist abnormally when compared to term born controls. This alteration in subplate involution associated with preterm birth may be due to more subtle forms of injury. The consequences of these alterations in subplate evolution on cortical development are unknown. However cortical gyration is reduced in the preterm brain at term equivalence, in comparison with normal term born control brains. However, MR cortical maturation at term corrected age is delayed in preterm babies with mild white matter abnormalities compared to those without (Fig. 127.7) [26–28].

In a rodent animal model of perinatal HI, the subplate neurons appear to be selectively vulnerable and their loss corresponds to the severity of the insult [29]. Investigators speculated that the glutamate receptor played a role in the pathogenesis, although the problem seems further complicated by more recent observations, suggesting a novel mechanism for subplate vulnerability.

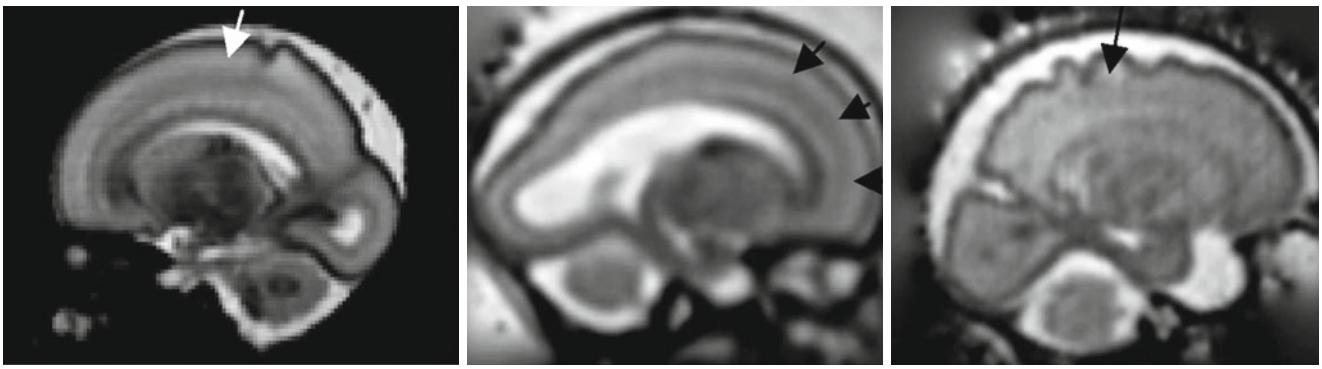

Fig. 127.5 Subplate visualization. T2 weighted images. Preterm infant 26+2 imaged at 28+1 weeks post-menstrual age. The subplate can be seen as a band of high signal intensity adjacent to the cortex (*arrows*)

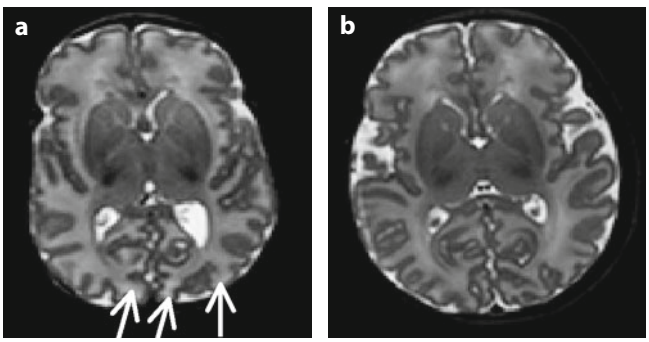

Fig. 127.6 Subplate involution. Preterm infant born at 26+3 and imaged at term (**a**). Term born control infant (**b**). Preterm infants at term have more subplate remnants, seen as regions of high SI adjacent to the gyral cortex (*arrows*) than term born controls, suggesting abnormal or delayed involution of the subplate layer

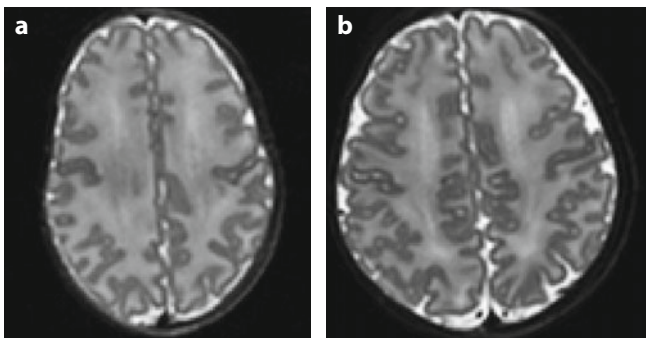

Fig. 127.7 Cortical development in the preterm. **a** Preterm infant born at 26+3 weeks gestation and imaged at term age. **b** Term born control infant T2 weighted images acquired in the transverse plane, at the level of the centrum semiovale. Preterm infants imaged at term age have less complex cortical folding than the term born controls [26, 27]

It is, however, well known that selective ablation of subplate neurons at critical time periods in development may cause altered cortical organisation [29, 30]. Thus, isolated injury to the subplate neurons, which play a fundamental role in axonal routing to and from the developing cortex, may result in abnormal thalamocortical connectivity. It has been speculated that this may explain the visual and somatosensory impairment seen in prematurely born humans [31]. Seventy-one percent of premature infants with moderate periventricular leukomalacia (PVL) during the neonatal period were found to have at least one abnormality of visual testing at 1 year of age, and yet 66% of these children had normal optic radiations, and all had a visual cortex that appeared normal [32]. Primary abnormalities within the visual cortex of subcortical white matter have not generally been demonstrated by imaging studies of infants with PVL, although the accelerated disappearance of the subplate may be a sign of injury. Cortical abnormalities in terms of loss of volume have been documented following PVL [33], but no studies to date have looked specifically at the visual cortex. The neurobiological basis for their visual impairment is therefore not well understood and abnormalities in the visual cortical function may occur as a secondary phenomenon due to injury elsewhere. In more recent studies, Ricci [34] and colleagues reported an association with poor visual function and thalamic atrophy. However, others [35] have suggested a plausible explanation for failing visual test during the neonatal age period is that an unspecific parenchymal lesion may damage the complex connectivity network as [34–36].

The cortical subplate continues to be related to brain development even late in gestation. In newborn babies at term with HIE, potential WM involvement may occur due to subcortical laminar necrosis in the subcortical subplate, as shown by highlighting in areas close to the cortex, which is particularly evident at the depths of sulci. The predilection of cortical necrosis at the bottom of sulci remains unexplained, although the fact that the subplate first disappears at the depths of sulci as the cortex folds may indicate an increasing vulnerability of the local resident neurons as they are more mature than those at the tops of gyri.

The role of subplate neuronal injury as a neurological basis for later impairments needs to be further investigated. A more sophisticated *in vivo* imaging approach such as serial diffusion tensor imaging studies is needed [24, 37].

127.3.3 The Developing White Matter

The association of preterm delivery and white matter injury in the form of periventricular leucomalacia (PVL) has been recognised since the 1960s [38]. Imaging studies have emphasised that injury to the developing white matter is associated with secondary abnormalities involving the entire thalamo-cortical circuit, giving rise to thalamic atrophy and a decrease in cortical volume [33]. Recent MR studies have also identified a spectrum of WM abnormalities in the preterm brain. PVL represents the most severe, with new findings of punctuate WM lesions and the diffuse appearance of DEHSI being reported more recently [3].

127.3.4 Oligodendrocytes

The vulnerability of preterm white matter appears to be multifactorial, relating to vessel anatomy with arterial end and border zone regions of relatively poor perfusion, low baseline blood flow, impaired regulation of blood flow in the sick infant and inherent susceptibilities of pre-oligodendrocytes (preOLs) to injury [6, 39]. The current theory is that both hypoxia-ischemia and infection lead to microglia activation that in turn leads to cytokine release, production of free radicals with both reactive oxygen and reactive nitrogen species and glutamate release, all of which contribute to death of the immature oligodendrocyte which demonstrate a vulnerability to oxidative stress [40] and glutamate receptor immaturity [41].

The ultimate result of either event would be a deficit of mature oligodendroglia, and a consequent impairment of myelination, the hallmark of PVL. Early white matter injury may result in a combination of delayed preOL degeneration and preOL maturation arrest. The persistence of susceptible populations of preOLs renders chronic white matter lesions markedly more vulnerable to recurrent hypoxia-ischemia. PreOL maturation arrest may predispose to more severe white matter injury in preterm survivors that sustain recurrent hypoxia-ischemia [42].

127.3.5 Axons

Axonal injury has been recognized for many years to be a feature of the focal necrotic component of PVL. Perhaps more important quantitatively, axonal degeneration, detected by the apoptotic marker fractin, has been recently found to be a feature of the diffuse component of human PVL. Whether the axonal degeneration observed in diffuse PVL is a primary injury or a secondary effect remains unclear. However, if primary axonal injury did occur, the expected results would be hypomyelination via failure of axonal-oligodendroglial interactions and decreased cortical [33] and thalamic/basal ganglia volumes [43, 44].

127.3.6 Thalami

We have included thalami in this section due to direct links with white matter. Recent human neuropathological data have demonstrated a high incidence of damage to specifically mediodorsal and reticular nuclei of the thalami in infants with PVL. Four patterns may be considered: diffuse gliosis, status marmoratus, micro-infarcts, and macro-infarcts. Irrespective

of the pattern and/or mechanism, it has been suggested that the reduced thalamic volumes revealed by neuroimaging are caused by neuronal loss [45]. Thalamic neuronal loss would be consistent with either primary injury and/or secondary anterograde and retrograde trophic effects. If there is primary neuronal injury, secondary effects would involve white matter axons, with subsequent hypomyelination and impaired development of the cerebral cortex, and such changes would be expected to compound the effects of the initial WM injury characteristic of PVL.

The role of the cerebral cortex in the cognitive deficits in preterm survivors is poorly understood. Periventricular leukomalacia (PVL), the key feature of the encephalopathy of prematurity, is characterized by periventricular necrotic foci and diffuse gliosis in the surrounding cerebral white matter. A recent study tested the hypothesis that reductions in the density of layer I neurons and/or pyramidal neurons in layers III and/or V are associated with PVL, indicating cortical pathology potentially associated with cognitive deficits in long-term survivors. In 15 controls (23 gestational weeks to 18 postnatal months), there was no difference in pyramidal density among incipient Brodmann areas, suggesting that cytoarchitectonic differences across functional areas are not fully mature during the fetal and infant periods. There was a marked reduction (38%) in the density of layer V neurons in all areas sampled in children with PVL cases compared with controls, in whom the six-layer cortex was visually distinct ($P < 0.024$). This may reflect a dying-back loss of somata complicating transection of layer V axons projecting through the necrosis in the underlying white matter. This study emphasised the potential role of secondary cortical injury in the encephalopathy of prematurity [46].

127.3.7 The Role of Microglia in WM Injury

Microglia are the main cell type implicated in injuries to the developing WM. Microglia are hemopoietic in origin and migrate into the brain from approximately 6 weeks' gestation. There are at least three morphological forms (amoeboidal, activated and ramified), which are distinguished by staining with markers such as tomato lectin and Iba. In the normal adult brain, the majority of microglia are ramified or resting, and activated microglia are assumed to be pathological. The normal immature brain contains all recognized morphological forms of microglia (ramified, activated and amoeboidal), although the exact site and numbers of the different forms alters with increasing gestation. Normal developmental or resident microglia perform many essential roles. These include the phagocytosis of unwanted tissue from programmed cell death as shown by labelling of microglia and apoptotic cells in regions of synaptogenesis and neuronal differentiation [47]. Resident activated microglia are also involved in brain modelling by secreting growth factors such as nerve growth factor (NGF), basic fibroblast growth factor (bFGF) and neurotrophin-3 [48], which provide trophic support for neurons and glia cells.

There is a maturation dependent concentration of microglia in apparently normal cerebral white matter during the third trimester of human gestation [49]. A few of these microglia are MHC II immunopositive cells, further evidence that they are developmentally activated resident cells and not immune induced. It has been suggested that the presence of numerous resident microglia in the immature brain increases its susceptibility to WM injury. Microglia co-exist during this developmental period with pre-oligodendrocytes which, as discussed, are also particularly susceptible to ischemic and inflammatory injury. Microglia are known to play a pivotal role in diffuse white matter injuries in the immature brain such as PVL.

Microglia are a potent source of both inflammatory cytokines and free radicals. Reactive oxygen and nitrogen species (ROS/RNS) release is mediated via Toll-like receptors known to be present on microglia. Interferon γ expression has been demonstrated in astrocytes in diffuse PVL. Interferon γ toxicity is greater for pre-OLs than for mature cells and is potentiated by tumor necrosis factor alpha (TNFα), which is produced by microglia. Evidence for the contribution of inflammation to preterm white matter injury emanates from studies of pregnant and neonatal animal models, where responses to exogenous lipopolysaccharide (LPS) are particularly involved. These studies have identified striking upregulation of inflammatory cytokines and microglial activation. LPS activates the innate immune system through an interaction with a specific toll-like receptor (TLR4) on immune cells, including microglia. Further evidence supporting the role of microglia in WM injury is provided by many studies using animal models of WM injury which demonstrate a decrease in injury in the presence of inhibitors of microglial activation such as chloroquine, minocycline and melatonin. Strategies for decreasing immune activated microglial activity in response to injurious processes should ideally not interfere with the function of normal developmentally active resident microglia.

In vivo and *in vitro* imaging studies of the immature brain have attributed regions of altered signal intensity within the developing white matter to the presence of dense clusters of microglia [50]. An increase in activated microglia in all periventricular white matter areas (sometimes called WM crossroads) compared to the deep white matter and subplate has been demonstrated in a cohort of 23 human brains with gestational ages of 22 to 40 weeks (Fig. 127.8). In addition, these authors demonstrated increased numbers of periventricular microglia in association with germinal matrix/intraventricular hemorrhage [51]. Although the morphological appearances of Iba positive cells do not differentiate normal developmentally activated microglia from abnormal immunoactivated microglia, some differentiation between developmentally activated microglia and immune/injury activated microglia may be made based on their site and number and the presence of overt tissue injury. However, functional differentiation may require more specific

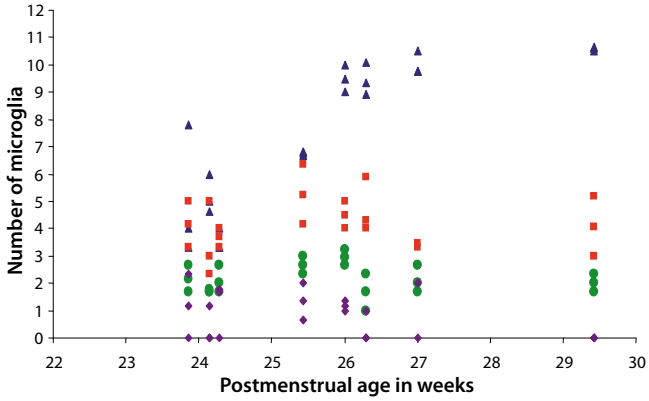

Fig. 127.8 Distribution of Iba 1 immunopositive microglia in the frontal, occipital and parietal lobes of the extremely preterm normal brain. Microglia numbers at the PVWM (*blue*) were significantly higher than deep white matter ($p = 0.0251$; *red*), subplate ($p < 0.0001$; *green*) and cortex ($p < 0.0001$; *purple*). This difference was most marked over the gestational age range in which PVL is most common

immunological markers. For example, mouse models have shown that in response to ibotenate, early microglial activation was CD45 negative, i.e., these cells are likely to have originated from the normal resident population [52].

In vitro studies of animal models have shown that the LPS-induced inflammatory response initiated by microglia was mediated via the TLR4 *My*D88-dependent pathway [53, 54]. This activation of microglia causes neuronal death, whilst *My*D88-deficient microglia do not. Manipulation of this activation may provide an opportunity for neuroprotective intervention. In a separate rodent model, LPS preconditioning was shown to have an altered protective response to subsequent ischemic injury via the TRIF-IRF3-IFNβ TLR4 cascade [55], which may have a beneficial effect on microglia. Investigation of these TLR4 activation pathways would enable functional characterization of different microglial phenotypes in the immature human brain, especially in association with lesions detected by MR imaging. In addition, co-labelling for apoptosis and for axonal injury would determine any deleterious effect by immune activated microglia.

127.3.8 The Germinal Matrix

The term germinal matrix is used by neonatologists to describe dense regions of tissue seen primarily within the caudothalamic notch on cranial ultrasound. On MR imaging scans of younger babies similar areas are also seen in the roof of the temporal horn and in the frontal areas externally adjacent to the frontal horns. These areas correspond to the ganglionic eminences referred to by neurobiologists. The germinal layer lining the entire ventricle may be identified on MR imaging of the fetal or very preterm brain but is not seen on ultrasound. In the fetal brain this layer is very prominent, but in infants born preterm, involution is already evident. On MR imaging of postmortem specimens, the germinal layer increases exponentially; it reaches a maximum at around 23 weeks' PMA and then decreases dramatically [56].

The germinal and subventricular layers are sites of neuronal and glial cell proliferation and migration. The characteristic vulnerabilities of early migrating neurons and preoligodendrocytes to injury have already been discussed, but their site of origin, the germinal matrix itself, is a common site of injury in the preterm brain. It is vulnerable to both hemorrhagic injury, as a result of the dense vascular network, and to injury from infection, e.g. by cytomegalovirus, which results in necrosis and cyst formation.

Germinal matrix intraventricular hemorrhage (GMH/IVH) has decreased in incidence because of improvements in intensive care, specifically in respiratory morbidity with the introduction of antenatal steroids for threatened preterm labor and artificial surfactant at the time of delivery. However, with the survival of the most extremely preterm infants who remain very vulnerable, germinal matrix hemorrhage remains a major cause of morbidity and mortality. The incidence in those born at 24–26 weeks' gestation or below 750 g birthweight is approximately 20–30%. The inverse relationship with gestational age indicates that the larger the matrix, the more susceptible it is to hemorrhage. In the term born neonate in contrast, intraventricular hemorrhage is thought to originate from the choroid plexus and is recognized as being a complication of sinus thrombosis particularly when associated with thalamic hemorrhage [57]. A germinal matrix hemorrhage can also derive from sinus thrombosis in late-preterm babies [58]. The site of hemorrhage in the preterm is usually from the ganglionic eminence of the caudothalamic notch or less frequently in the temporal horn [59]. These regions have a rich capillary network, which is vulnerable to rupture and hemorrhage because of the poor vascular integrity of involuting immature vessels and inadequate connective tissue support with a deficiency in mesenchymal and glial elements. Furthermore, a pressure passive circulation, compliant skull and disturbances in coagulation in the sick preterm exacerbate the fragility of the matrix. Hemorrhagic lesions are thought to be secondary to venous congestion with distortion and tearing of local small venous tributaries by the presence of blood in the perivenous space [60–62]. The hemorrhage originates and destroys the germinal matrix and this may impair proliferation and late migration of GABAergic interneurons to upper cortical layers, and the thalamus could contribute to defective cortical and thalamic development. There is some neuropathological data to support this [63], although it is difficult to determine the independent effect of progenitor cell loss on subsequent brain development because infants that die are likely to have additional brain lesions. Such complications of GMH/IVH include venous infarction and ventricular dilation, both of which will additionally injure developing white matter and

possibly thalamic tissue and therefore disrupt thalamo-cortical connectivity and cortical development. So, whilst there is likely to be a disturbance of the late GABAergic neuronal proliferation and migration from the subventricular zone (SVZ) and the ganglionic eminence (GE) to upper layers of the cerebral cortex and from the GE to the thalamus, venous infarction will cause additional destruction of both axons and pre-OLs, resulting in the formation of a porencephalic cyst. Thalamocortical connections will be disrupted, resulting in thalamic atrophy and overlying cortical development may be impaired. Thalamo-cortical disconnection may be demonstrated at a distance from the site of infarction.

The neurodevelopmental consequences of this are evident in follow-up studies such as the DRIFT trial, which enrolled neonates with marked ventricular dilation complicating GMH/IVH. The majority also had evidence of venous infarction. While the trial demonstrated some improvement in neurodevelopmental outcome following specific intervention, as a study group these infants with complications showed severe neurodevelopmental impairments at 2 years corrected age [64]. A secondary imaging study of total brain tissue volume and cerebellar volume highlighted the need to preserve supratentorial tissue and avoid cerebellar compression by a dilated fourth ventricle [65]. Reduced cerebellar size following GMH/IVH may be multifactorial and related to a primary cerebellar hemorrhage or to secondary atrophy as a consequence of supratentorial lesions [43, 44].

127.3.9 The Cerebellum in Extremely Preterm Babies

Primary cerebellar hemorrhage arising from the external granular layer and associated tissue infarction is relatively common in very preterm infants. There is a well documented association between intraventricular and cerebellar hemorrhage. In one large ultrasound study of 1242 preterm infants with intraventricular hemorrhage, 77% of infants also had cerebellar hemorrhages [66]. MR imaging studies seem to confirm this high vulnerability of the most premature babies to develop cerebellar hemorrhage [67].

A study using the mastoid window for better visualization of the posterior fossa demonstrated an incidence of cerebellar hemorrhage in 3% of preterm infants <1500 g, with nearly 60% of these occurring in infants less than 750 g [66]. In the very preterm infant, the hemorrhage originates within the hemisphere with involvement initially of the subpial and subependymal layers sites of the germinal matrices and subventricular zones respectively. The more extensive lesions extend and involve both the cerebellar cortex and white matter. In contrast, in the term infant the site of origin is more frequently the vermis. Pathogenesis in the preterm infant shares many similarities with the causes of intraventricular hemorrhage. In the term infant, trauma is a more important factor. Injury to the developing cerebellum will result in disruption of neuronal migration, which continues into infancy in the cerebellum with consequent cerebellar disconnectivity.

The role of the cerebellum in cognitive function is being increasingly recognized. It is not surprising therefore that 40% survivors of preterm cerebellar hemorrhage demonstrate cognitive deficits and 37% have autistic spectrum disorders [68]. In term infants, subsequent motor impairments are described more frequently, particularly in association with larger lesions [69, 70].

127.3.10 Effects of Germinal Matrix Intraventricular Hemorrhage

There is evidence to suggest that less severe forms of GMH/IVH may be associated with periventricular WM injury. This may result in milder degrees of ventricular dilation and studies have reported an association with later cognitive function in such infants [71, 72]. In addition, two studies have reported worse outcomes in survivors of preterm birth where subsequent ventricular dilation was associated with an original GMH/IVH [73, 74]. In the rabbit kit, IVH was associated with an increase in periventricular white matter (PVWM) microglial activation and axonal disruption and apoptosis [52]. An increase in activated microglia has also been demonstrated in human PVWM in the presence of uncomplicated IVH (Fig. 127.9). Tissue injury may occur secondary to free radical release, secondary to the presence of free iron from the blood and the presence of resident activated microglia may exacerbate this process.

It is unclear whether as the germinal matrix involutes it becomes less susceptible to the effects of infection. The development of subependymal cysts in the persisting germinal matrix of the caudothalamic groove postnatally suggests that the tissue remains vulnerable to viral infection in more mature infants, but the consequences for progenitor cell destruction may be less severe as the infant matures.

127.4 The Late Preterm Infant

In recent years the vulnerability of late preterm infants of gestation between 34 and 37 weeks has become recognized. Reports demonstrate increased short-term neonatal morbidity in low risk late preterm pregnancies [75, 76] with a 12-fold increase in mortality compared with term born controls and a mortality rate of 0.8% [77]. Studies of surviving late preterm infants have conflicting results, with no significant differences in childhood outcome [78]. A study at school entry showed that the risk for developmental delay or disability was 36% higher among late preterm infants compared with term infants [79]. However, outcome studies at 12 and 18 months stress the importance of correcting for gestational age when interpreting results [80].

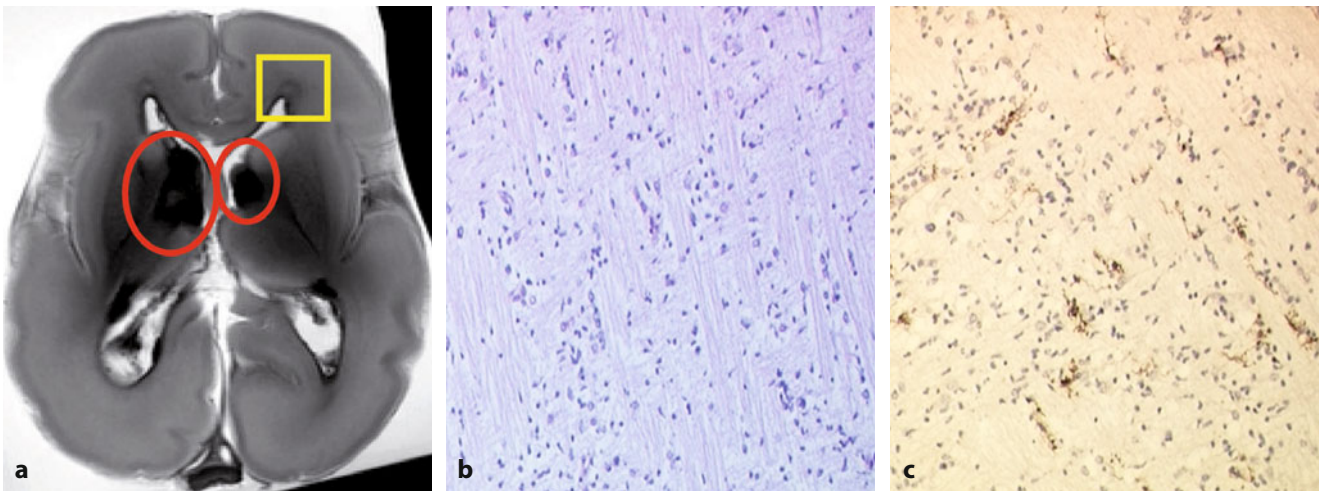

Fig. 127.9 T2 weighted MRI image (**a**) of a preterm infant born at 26 weeks and imaged postmortem at 28 weeks. There is bilateral germinal matrix/intraventricular hemorrhage seen as foci of low signal intensity (red circles). PAS (**b**) and Iba (**c**) staining through anterior periventricular white matter, (in the region of the yellow rectangle) showing intersecting axons, so called WM crossroads (**b**). Many of the cells in this region are microglia (**c**), which have roles associated with axonal guidance and WM modeling. Their presence may enhance the susceptibility of these regions to injury

Neuromorbidity in this group has been attributed to many factors. The cellular pathology of PVL and the developmental characteristics of oligodendrocytes and neurons put the late preterm brain at risk of injury. The cortical volume in the very preterm infant at 28 gestational weeks is 13% of term volume [11]. The cortical volume in the late preterm infant is only 53% of the term volume, with approximately half the volume being attained in the last 6 weeks before 40 gestational weeks. In addition, minimal myelinated white matter is present in the very preterm infant (around 29 weeks), but increases dramatically in volume as term is approached, with a five-fold increase between 35 and 41 weeks [81].

Studies of HIE in the preterm usually only include mature preterms and demonstrate that whilst mature preterms sustain injury to the central gray matter, the sites may differ to the more mature term brain. Mature preterm infants are more likely to show thalamic and globus pallidum lesions. In addition, they are more likely to sustain brainstem lesions [2]. This may be because of more severe injury or indicate a particular susceptibility of the brain stem at these slightly younger gestations [82].

It is of interest that the late preterm infant does not demonstrate cortical abnormalities around the central sulcus, which is a frequent abnormality n the term infant. This region is probably vulnerable due to the presence of myelination, which does not occur until approximately term [2].

127.5 The Term Brain

Studies of injury to the more mature term brain have concentrated on the vulnerability of the neuronal population. Imaging studies of neonates with perinatal injury complicating an acute hypoxic ischemic event demonstrate involvement of the cortex, central gray matter and brainstem. Vulnerability to specific neuronal populations in the term brain is due to multiple factors but, like white matter vulnerability in the preterm brain, excitotoxicity and oxidative stress play major roles in term injury. Vulnerability may be related to the maturational state of the neurons. There is over-expression of certain glutamate receptors in selective regions like the basal ganglia.

The N-methyl-D-aspartate (NMDA) glutamate receptor subtype is the predominant mediator of this type of injury because of its coupling to neuronal nitric oxide synthase-containing neurons in the postsynaptic density complex. The NMDA receptor can change subunit compositions with development of the NR2B subunit predominating early, followed by increasing expression of NR2A. NMDA receptors with NR2B seem to have a slower deactivation and higher conductance. Following an hypoxic insult, there are differential effects on NMDA receptor subunit composition and these effects differ by age. This interaction may result in the production of both nitrogen and oxygen free radicals that in turn injure nearby cells [83]. The vulnerability to neurons in the basal nuclei also appears to be related also to the local environment as neuronal nitric oxide synthase-containing neurons make neurons relatively resistant to severe hypoxic ischemia [84]. There is also an overabundance of NMDA receptors in this region at term allowing for the robust glutamatergic synapses necessary for long-term potentiation and connectivity but also allowing the neuron to be more vulnerable to glutamate attack. It has been suggested that these neurons may be protected either pharmacologically or by gene

knockout to render the region less vulnerable and prevent neuronal loss [85, 86].

Regions of vulnerability following acute hypoxic ischemia at term are recognized as being those that are metabolically active with increased energy requirements such as the basal ganglia and thalami [87]. Regions that are actively myelinating also appear to be more vulnerable. In the term infant, regions around the myelinating corticospinal tracts arising from the cortex at the central sulcus are frequently involved. However, in less mature infants these areas are often spared the metabolic demands of myelination, which may compound the additional vulnerability of cortical neurons, which express a high number of Ca^{2+} permeable glutamate receptors at term. This contrasts with the peak period for overexpression of these receptors in oligodendrocytes and subplate neurons, which is in the late second and early third trimesters.

127.6 Focal Infarction

White matter injury is often seen in the mature term brain, presumed either to be due to a vascular event relating to vessel obstruction by an embolus or by vessel spasm. Affected areas are as middle cerebral artery infarction or more global hypoperfusion such as in parasagittal or watershed injury. Bilateral injury is often associated with a history that suggests chronic hypoxia, e.g.decreased fetal movements, infection or hypoglycemia. Injury usually involves both the WM and the cortex. Thalamic involvement is common but may occur as a secondary phenomenon. Posterior WM may be involved more frequently than anterior WM regions, particularly in association with hypoglycemia. This may be explained in terms of watershed injury, with posterior regions representing the watershed areas for all three cerebral arteries. However posterior involvement with hypoglycemia does not always selectively involve borderzones between arterial territories.

The hypothesis of a chronic or repeated event priming the WM suggests that the WM is made progressively more vulnerable so that it can be injured by a relatively mild hypoxic-ischemic event. The additional role for infection in such term infants may again relate to a cascade of injurious events precipitated by a fetal inflammatory response. The underlying mechanism for this acquired increased vulnerability remains unclear, but it may involve altered mitochondrial function. When mature oligodendrocytes die after exposure to kainate, (1) AMPA receptors are the most important mediators, (2) kainate receptors play a smaller role, and (3) death occurs predominantly by necrosis, not apoptosis. [88].

127.7 Programmed Cell Death

Apoptosis is a critical component of normal brain development but, as the brain is poised to initiate programmed cell death around human birth, it becomes more susceptible to the initiation of a cell death pathway. Although necrosis plays a major role in early neuronal death in both the immature and mature brains after injury, there is a spectrum of cell death that includes apoptosis within the first 24 h following perinatal hypoxic ischemia [89]. Programmed cell death is a crucial mechanism in the control of the final number of neurons and glial cells. This process, commonly referred to as apoptosis, can be observed from the earliest gestational ages with different times of cell death peaks in different brain regions [90]. In addition to refining normal brain development and connectivity, the plasticity it provides may be important in repair mechanisms following injury. However, a predisposition to programmed cell death may be exploited following injury and result in an increase in cellular apoptosis in injured regions as a consequence of disturbances disrupted connectivity.

The concept of priming of a brain region to injury is an important one, although a region such as the white matter is not traditionally considered to be vulnerable. There is, however, animal evidence to suggest that such priming occurs with preinjury insults (i.e., delivery related events) such as hypoglycemia, intrauterine growth restriction (IUGR) and infection. The biological mechanisms by which such events increase the susceptibility of WM to injury are poorly understood but investigations of preconditioning may explain the acceleration of such events due to birth.

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Inflammation and Perinatal Brain Injury

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128.1 Introduction

Inflammation is a systemic and local immune reaction to injury secondary to microbial invasion or other damaging events like trauma and hypoxia-ischemia (Fig. 128.1). This response aids in identifying extrinsic pathogens and kills microbes (and affected cells) if the injury is caused by infection [1]. Irrespective of the primary triggering event, inflammation often causes brain damage during its acute stage (collateral damage) followed by a secondary phase that in most cases promotes tissue repair and regeneration [2].

Historically, several findings support a link between brain injury and antenatal/postnatal inflammation [3]. In the 70s it was discovered that postnatal sepsis was strongly associated with brain injury in spite of the lack of microbial invasion in the brain parenchyma [4]. Induction of sterile systemic inflammation in newborn kittens with lipopolysaccharide (LPS, fragment of the gram-negative bacterial cell wall) induced white matter injury [5]. These studies suggested that infection triggers a systemic inflammatory response that inflicts brain injury under some conditions. During the 60–70ties it was discovered that cytokines, a family of ubiquitous and pleiotropic immune molecules with pro-, anti- or immunomodulatory properties orchestrate the inflammatory response [6]. Indeed, cytokines were shown to play a critical role in preterm delivery [7] and initiation of a fetal inflammatory response with subsequent development of brain injury and cerebral palsy [8, 9].

In parallel, experimental work demonstrated that non-infectious insults like hypoxia-ischemia and excitotoxicity elicited cytokine expression [10] and activation of immunoinflammatory microglia/macrophages [11, 12] in the immature brain that seemed to play a role for development of brain injury [13–15]. Epidemiological studies found that chorioamnionitis was associated with cerebral palsy in term infants [16] and there was a remarkable correlation between cytokine levels 2.5 days after birth and cerebral palsy in mostly term infants [17].

These early studies indicated that cytokine-mediated inflammation is an important factor for development of brain injury irrespective of whether the inflammatory response was caused by infection, hypoxia-ischemia or excitotoxicity. However, since these early reports the complexity, diversity and sometimes contradictory (Jekyll-Hyde) properties of the inflammatory reaction have become increasingly clear. Furthermore, cytokines have been discovered to have other roles, e.g., in brain development and in the setting of CNS vulnerability as well as in neuronal and oligodendroglial regeneration [18]. In this short review we will summarize some of the recent pertinent experimental and clinical findings.

128.2 Immune Molecules and Brain Development

Molecules classically involved in immune responses are pleiotropic and have critical non-immune functions during normal CNS development, including neuronal and glial cell migration, cell differentiation, axonal path finding and synaptic plasticity. These molecules include classical pro-inflammatory cytokines and chemokines as well as proteins of both the innate and adaptive immune system.

Cytokines are of importance during all stages of neural development. Members of the transforming growth factor beta (TGFβ) cytokine superfamily, including bone morphogenetic proteins (BMPs), are critical for neural induction and normal CNS development requires active inhibition of BMPs [19]. Later in CNS development, maintenance of the progenitor cell population is dependent on several members of the gp130 cytokine family, including leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) [20]. Also classical pro-inflammatory cytokines, such as interleukin-1 beta

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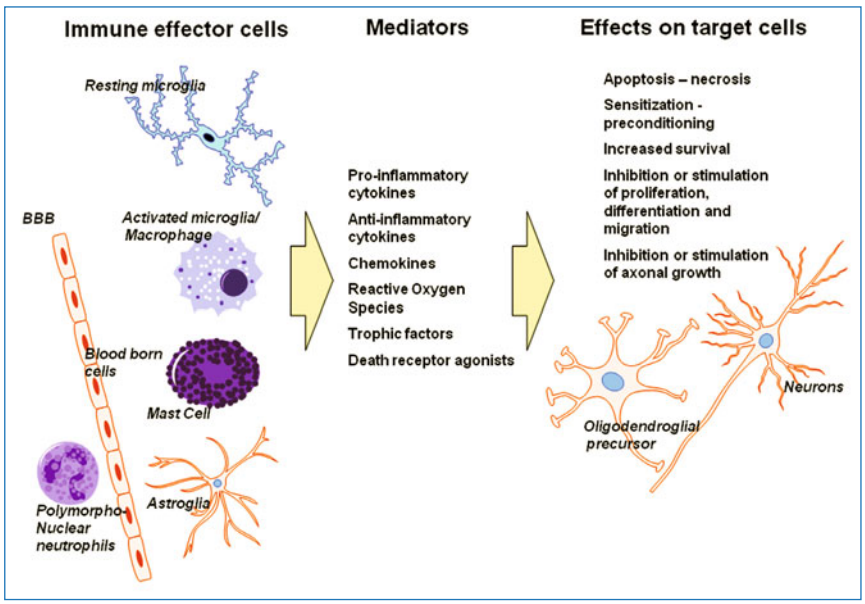

Fig. 128.1 Inflammation and perinatal brain injury. Infection, hypoxia-ischemia or other insults activate immune effector cells in the vascular compartment as well as in the brain parenchyma. These cells produce a plethora of mediators that exert protective as well as toxic effects in the immature CNS

(IL-1β), have been reported to modulate neuronal and glial cell survival during development [21] and to regulate progenitor proliferation [22]. There is also evidence to suggest that IL-1β promotes differentiation of oligodendrocyte progenitors as well as maturation and survival of differentiating oligodendrocytes [23].

Chemokines are traditionally considered as secreted leukocyte chemoattractants. However, several chemokines and their respective receptors are constitutively expressed in the CNS and significant evidence supports chemokines, such as CXCL12, as key regulators of early brain development, including survival and migration of neural and oligodendrocyte precursors [24–26]. CXCL12, via activation of its receptor CXCR4, is also crucial for tangential migration of Cajal-Retzius cells and thereby the normal development of the cerebral cortex [27].

Innate immune responses have been implicated in normal CNS development. Both C1q and C3, components of the classical complement cascade, are expressed in neurons in the developing brain and play an important role in synapse pruning in the visual system during development [28] and complement-derived C3a regulates differentiation and migration of neural progenitor cells [29]. Toll-like receptors (TLRs) belong to a recently discovered family of innate immune receptors [30]. TLRs are expressed in the developing brain and stimulation of TLR3 negatively regulates both axonal growth in sensory neurons [31] and embryonic neural progenitor cell proliferation [32]. Furthermore, TLR8 is expressed in neurons and axons during mouse development and stimulation of TLR8 in cultured cortical neurons causes inhibition of neurite outgrowth and induces apoptosis [33]. In addition, TLRs are expressed in adult neural progenitor cells and may influence their proliferation and differentiation as TLR2 deficiency impaired hippocampal neurogenesis, whereas the absence of TLR4 resulted in enhanced proliferation and neuronal differentiation [34]. Also the adaptive immune system, including the class I MHC molecules has been shown to play a role during normal development and plasticity [35].

128.3 Inflammation and Brain Injury in Preterm Infants

Infection/inflammation is strongly associated with preterm delivery and accounts for 20–40% of all cases with preterm birth [36] including the majority of cases with spontaneous preterm birth before 30 weeks of gestation, i.e., those at highest risk of neurological morbidity [37, 36]. Hypothetically, ascending microbes activate TLRs leading to increased accumulation of polymorphonuclear neutrophils and expression of pro-inflammatory cytokines (IL-6, IL-8, IL-1β, TNF-α) in the decidua and chorioamniotic membranes histological chorioamnionitis (HCA). The inflammatory response elicits myometrial contractions, cervical ripening and preterm prelabor rupture of the membranes (PPROM) subsequently resulting in preterm delivery [38]. In most cases, chorioamnionitis (CA) is subclinical and manifests rarely as clinical chorioamnionitis (CCA) (maternal fever, tachycardia, uterine tenderness, malodorous amniotic fluid and leukocytosis/raised C-reactive protein). CA is a predominately fetal infection and spread of the inflammation and/or microbial invasion from the membranes to amniotic fluid to umbilical cord to fetal blood (fetal inflammatory response syndrome) increases, not unexpectedly, the risks of fetal morbidity and mortality see below [39, 36]. The degree of

fetal inflammation (defined as e.g. IL-6 elevation in amniotic fluid or blood) determines at least acute neonatal morbidity, whereas detection of microbial invasion does not seem to aggravate outcome further [39].

Some data indicate that intrauterine inflammation is associated with neurological injury. Severe brain lesions were 10 times more common after spontaneous onset of labor (with high occurrence of CA) than in physician-initiated delivery (with a low occurrence of CA) at a comparable gestational age offering support of the inflammatory hypothesis [40]. A similar, but not as marked, correlation was found between diplegic cerebral palsy (CP) and type of delivery onset [41], whereas no such association was found by Jacobsson et al [42]. In a very important study, the presence of umbilical cord inflammation (funisitis) or high amniotic IL-6 and IL-8 levels conferred an increased risk of CP in the offspring at 3 years of age even after adjustment for differences in gestational age [9].The proposal that fetal rather than maternal involvement predicts neurological outcome is supported also by a large study (1078 infants, 500–1500g, 47 with sonographic echolucency) showing that fetal vasculitis was associated with an increased risk of late echolucent white matter injury in cases born shortly after PPROM (OR 10.1, CI 1.03–114) [43]. Furthermore, cytokines and CD45RO(+) T lymphocytes in fetal blood predicted cerebral white matter injury detected by magnetic resonance imaging soon after birth [44].

According to a meta-analysis, CCA is associated with CP (RR 1.9, CI 1.4–2.5) and cystic periventricular leukomalacia (PVL) (3.0; 2.2–4.0), whereas CA constitutes a risk factor for PVL (2.1; 1.5–2.9) but not CP (1.6; 0.9–2.7) [45]. However, a strong association was found between polymicrogyria (a migratory disturbance of the cortical plate/cerebral cortex) and CA [46]. The brains of fetuses at gestational age 15–26 weeks in the context of CA were compared with those aborted for other reasons. Polymicrogyria was found in 25/32 cases (78%) with infection compared with none in the control group. These remarkable results suggest that inflammation inflicts on brain development (see above), which agree with experimental studies in adult rodents [47] and with a recently published review suggesting that microglial activation may disturb cortical migration and stem cell survival [48].

Moreover, several investigators have shown that intravenous administration of LPS to fetal sheep [49–52] or into the amniotic fluid [53, 54] results in white matter damage. Early studies suggested that the LPS caused brain damage by reducing cerebral blood flow [55, 56], however more recent evidence, using lower doses of LPS, do not find that perfusion deficits contribute to injury [57, 58].

It is important to mention that not all studies support the inflammatory hypothesis. For example, no association between white matter injury/neurological outcome and HCA or umbilical blood IL-6 were found by Kaukola et al [59]. Reiman et al [60] found no correlation between fetal or maternal HCA and brain injury defined by ultrasound or magnetic resonance imaging (MRI), which agree with Chau et al [61], who found no association between fetal or maternal HCA and MRI-defined brain abnormalities in preterm infants at 24–32 weeks of gestation. Furthermore, Kumazaki et al [62] detected no difference in ultrasound-defined PVL in cases with versus without fetal or maternal HCA.

These discrepancies cannot easily be explained but generally there is a lack of statistical power in many studies (both those pro and con the inflammatory hypothesis), inconsistencies in the definition of CA (see above), differences in cases included, study design or the fact that inflammation has many different phenotypes (toxic, beneficial, no effect) depending on timing and other circumstances (Fig. 128.1).

There are several clinical and experimental studies indicating that CA may be a stronger risk factor if combined with another exposure, the so-called multiple-hit hypothesis [63]. For example, HCA alone was not a risk factor but if combined with signs of a placental perfusion defect the risk of abnormal neurological outcome was increased (3/10 with HCA versus 3/43 without HCA) [59]. In another study, culture positive postnatal infection increased the risk of MRI-defined brain injury if occurring in combination with hypotension [61]. Indeed, chorioamnionitis (with or without funisitis) increases the risk of hypotension in very low birth infants [64].

128.4 Inflammation in Term Brain Injury

Inflammation has been extensively studied as part of the secondary response to experimental hypoxic-ischemic (HI) injury and the presence of an inflammatory response has been demonstrated also after clinical HI. There are, however, also studies that suggest that infection/inflammation may precede brain injury or sensitize to subsequent HI. In addition, the inflammatory response and its involvement in injury and repair in the immature brain is dependent on timing as well as context and differs substantially from that seen in the adult brain.

128.4.1 Clinical Findings of Inflammatory Factors in Term Brain Injury

Several studies demonstrate that an inflammatory response is elicited in CSF from term infants fulfilling criteria for acute intrapartum asphyxia. Elevated levels of pro-inflammatory cytokines IL-6 and IL-8 in CSF are correlated with severity of encephalopathy as well as neurodevelopmental outcome [65, 66]. In samples obtained within 24h after birth IL-1β and TNF-α show similar correlations [67, 68] and comparisons with serum levels suggest that the cytokines are produced within the brain [69, 68]. Support of cytokines contributing to brain injury rather than just being biomarkers of inflammation is presented in a study showing increased risk for CP in infants with specific IL-6 genotype [70].

The correlation between brain injury and systemic inflammation is less well documented, but at least one study show that asphyxiated infants with adverse outcome had elevated pro-inflammatory cytokines in serum [71]. An intriguing study of dried blood spots from term infants who later developed spastic CP demonstrates, however, elevated levels of multiple cytokines in virtually all affected infants in comparison with term matched controls even if HI is likely to be the cause of only a minor fraction of the CP cases in that study [17].

The suggestion that exposure to infection or inflammation in itself or together with HI may lead to term brain injury is further supported by individual studies [16] as well as a large meta-analysis showing that clinical chorioamnionitis is an independent risk factor (OR 4.1) for term/near-term CP [72]. In the same analysis, chorioamnionitis in the mother was also associated (OR around 5) with signs of neonatal depression (APGAR at 5 min <7, clinical diagnosis of birth asphyxia) as well as signs of neonatal encephalopathy (seizures) [72]. Since data on metabolic acidosis and from neuroimaging were not available it cannot be excluded that these findings were secondary to exposure to chorioamnionitis only. Extensive MRI studies show, however, that a large majority of term infants with the combination of neonatal depression and neonatal encephalopathy have acutely evolving injuries suggesting intrapartum asphyxia [73]. It is thus likely that at least some of the infants with subsequent CP were exposed to chorioamnionitis as well as HI, giving additional support to the multiple-hit hypothesis. Further evidence of the detrimental effect of fetal inflammation in combination with HI is provided by a study of term spastic CP showing relatively low OR for exposure to chorioamnionitis as well as potentially asphyxiating factors (placental and cord complication) while the combination of both increased the risk for severe spastic CP hundred-fold [74]. In addition, neonatal serum cytokines are elevated after exposure to chorioamnionitis but the increase is most pronounced in infants who also fulfil very strict criteria for hypoxic-ischemic encephalopathy (including metabolic acidosis, moderate-severe encephalopathy and multiorgan failure), illustrating the close relationship between exposure to infection and clinical HI [75].

128.4.2 Experimental Studies Supporting Inflammatory Factors in Post HI Injury

The inflammatory response after experimental HI includes an early but restricted accumulation of neutrophil cells, mainly in blood vessels adjacent to injury [76, 77]. Resident microglia and/or invading macrophages are, however, the predominant inflammatory cell type after neonatal HI and infiltration of activated cells is detected as early as 2–3 hours after injury, followed by a marked increase in the following days that persists for weeks [11, 77]. Inflammatory cells may contribute to injury by release of excitatory amino acids, pro-inflammatory cytokines, degrading enzymes and reactive oxygen species (ROS), but microglia can also produce neurotrophic or neuronal survival factors upon activation [78]. In the neonatal setting HI is accompanied by a massive change in the expression of inflammation-related genes with close to 150 genes differentially regulated at 2–72 hours after the insult including up-regulation of cytokines, chemokines, proteases and ROS-forming enzymes [79].

A number of experimental studies show that inflammatory cells and inflammatory mediators may contribute to injury, but the complexity of the inflammatory response is illustrated by conflicting data or results limited to certain contexts. In addition, several studies indicate that the inflammatory mechanisms related to the development of brain injury are substantially different in the immature compared with the adult brain and that inflammatory cells may also have protective properties.

Factors that may influence the results of anti-inflammatory interventions include means and timing of inhibition and degree of injury. As an example, the pro-inflammatory cytokine IL-1β is up-regulated at the mRNA as well as protein level after HI and soluble IL-1 receptor antagonist protects from HI injury in newborn rats [80, 14] while genetic inhibition of IL-1β or IL-1α/β combined confers no protection in newborn mice [81]. IL-18 is another cytokine that is up-regulated after HI in newborn mice and transgenic mice lacking functional IL-18 are protected from injury [82]. Genetic inhibition of interleukin-1 converting enzyme (ICE) that converts IL-1β as well as IL-18 to their active forms is, however, protective only after moderate but not severe HI [83]. A similar restriction is found for matrix metalloproteinase-9 (MMP-9), a tissue-degrading enzyme expressed by activated microglia, where genetic knock-out attenuates injury after moderate HI only [84].

The difference between the adult and immature brain is illustrated by findings after neutrophil depletion and inhibition of the enzyme NADPH-oxidase that is responsible for ROS formation in inflammatory cells. Neutrophil inhibition is protective after adult [85] as well as neonatal HI injury [76] but neutrophil infiltration is limited in the immature brain [77] and protection is only seen if the depletion is induced well before injury suggesting early effects on brain microcirculation [86]. Neutrophil depletion also results in reduced free radical formation in the adult brain [87] and mice lacking functional NADPH-oxidase have 40% reduction of infarction volume after adult stroke [88]. This is in stark contrast to the immature brain where genetic or pharmacological inhibition of NADPH-oxidase results in un-altered or even aggravated brain injury [89]. These findings imply maturity-related differences in the inflammatory response to injury and suggest that ROS may have protective properties in the developing brain.

Recently, the possible protective properties of inflammation and in particular microglia cells have come into focus. Exogenous microglia cells are protective in adult models of ischemic injury [90, 91] but studies of microglia inhibition with minocyclin after neonatal HI are contradictory

with reports of strong protective effect after HI in neonatal rats [92], worsening of injury after HI in newborn mice [93] and transient protection after neonatal stroke [94]. It has thus been suggested that intervention aimed at protecting the brain from inflammatory injury should modulate microglia phenotype rather than merely inhibit the inflammatory response or single inflammatory mediators.

In adult brain different microglia populations have been identified after stroke and resident proliferating microglia expressing insulin-like growth factor-1 (IGF-1) and galectin-3 are protective [95]. Such sub-populations of microglia are yet to be identified in the neonatal brain, but a recent study of the novel inflammatory marker galectin-3 illustrates the possibility of modulating the inflammatory response after HI [96]. Previous studies show that galectin-3 activates NADPH-oxidase [97] and augments inflammation by increased chemotaxis and decreased apoptosis in inflammatory cells [98, 99]. In the neonatal setting genetic inhibition of galectin-3 resulted in attenuated HI injury with no effect on apoptosis or growth factor expression. Instead galectin-3 deficient mice had increased microglia infiltration but decreased oxidative stress and MMP-9 expression, suggesting changes in microglia phenotype [96].

In addition, the protection and the changes in inflammatory response in galectin-3 deficient mice were more pronounced in male pups, suggesting that gender may also influence the immune response in the immature brain [96]. The gender differences at this early age are more likely to depend on genetic differences than hormonal factors [100] and opens new intriguing avenues of research.

128.5 Inflammation and CNS Vulnerability

As pointed out above, there is strong experimental evidence to show that infection/inflammation may act in synergy with other insults and aggravate brain injury. Studies in both rats and mice have shown that exposure to LPS, sensitizes the immature brain to subsequent HI [101–104] and results in extended cerebral damage and long-lasting memory and learning impairment [105]. Similar sensitizing effects are seen with excitotoxicity [15], where pro-inflammatory cytokines and IL-9 exacerbate excitotoxic lesions of the newborn murine neopallium [15]. The underlying mechanisms of sensitization are not well understood, however at least LPS-induced aggravation of injury is known to act in a TLR-4 and MyD88 dependent manner [106, 107]. Microglia cells appear to play an important role in the injury process as LPS-stimulated microglia are able to kill neurons and oligodendrocytes, at least when grown in culture [108, 109]. Oxidative stress may also be involved in LPS-induced sensitization as the free radical scavenger N-acetylcysteine (NAC) provided marked neuroprotection following LPS/HI insult in neonatal rats [110]. As mentioned above, inflammatory stimuli can under certain circumstances also protect the brain from further events, so called preconditioning or tolerance [111]. Although the mechanisms that are responsible for the switch from protection to injury are not well understood, it has been suggested that endotoxin-induced tolerance may be mediated by up-regulation of corticosterone in neonatal rats [112] and interestingly, the synthetic corticosteroid anti-inflammatory drug dexamethasone was able to prevent both structural brain damage and subsequent learning and memory deficits in neonatal rats exposed to LPS-induced HI injury [113].

128.6 Conclusions

There is today ample support to show that inflammation is involved in perinatal brain injury. Early studies provided support for the hypothesis that inflammation can aggravate brain injury during the acute phase both in the setting of infection and sterile insults like hypoxia-ischemia. Subsequent clinical and experimental work support the concept that the immunoinflammatory molecular network is complex and multifaceted with important roles in brain development as well as setting of CNS vulnerability, and during the acute and repair phases of injury. There is potential for neuroprotective immunomodulatory therapies to be developed, but we still need a more detailed knowledge about the basic mechanisms. Hence, the net effect of immune responses – protective versus beneficial – are often difficult to predict and depends on many factors, for example, which cells and mediators are activated, time point in relation to insult, molecular context, gender and developmental age.

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Normal and Abnormal Neurodevelopmental and Behavioral Outcomes of Very Low Birth Weight Infants

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129.1 Introduction

Advances over the past ten years in perinatal and neonatal management of very low birth weight (VLBW) infants (<1500 grams and <30 weeks' gestation) have resulted in a significant increase in survival of fragile high risk infants, including those with extremely low birth weight (ELBW) (<1000 grams) [1–10]. Therapeutic advances including the use of antenatal steroids [3, 4], surfactant for respiratory distress syndrome [11, 12], prophylactic indomethacin for prevention of intra ventricular hemorrhage [13], improved nutritional management, and new ventilatory techniques [14] have all contributed to improved survival especially for infants with birth weights less than 1000 grams [5–10]. These high risk preterm survivors have increased complex neonatal medical morbidities affecting all organ systems including lungs, gastrointestinal tract, kidneys and brain, and post-discharge morbidities including increased growth failure, neurologic, developmental, cognitive, neuropsychological, functional and behavioral sequelae [15–25].

Most tertiary care programs in the United States and all neonatal training programs currently include a requirement for fellow participation in the care and evaluation of discharged infants in a structured follow-up program. A workshop on the Follow-up Care of high risk infants sponsored by the National Institute of Child Health and Human Development (NICHD), the National Institute of Neurologic Disorders and Stroke and the Centers for Disease Control and Prevention held in 2002 established guidelines for the provision of Follow-up Services [21]. The workshop concluded that two primary areas of responsibility for Neonatal Follow-up Programs are surveillance and research.

Surveillance is indicated so that centers can systematically monitor and report on the care of high-risk infants during their hospitalization and their outcomes after discharge from the neonatal intensive care unit (NICU). The availability of a data management surveillance system for the NICU facilitates the ability to annually audit the safety and efficacy of interventions, monitor identified quality indicators for the individual NICU, summarize annual data on neonatal morbidities and summarize annual post-discharge outcomes such as rates of cerebral palsy (CP), mental retardation, developmental delay, blindness, hearing impairment, behavioral abnormalities, autism, learning disabilities, growth failure and medical morbidities. Information about center outcomes for specific conditions and by gestational age categories also allows staff caring for high-risk infants to counsel parents with regards to prognosis [26]. A follow-up clinic may also provide a mechanism for the provision of seamless comprehensive medical, psychosocial, and intervention services to NICU graduates [27]. Outcome data can be shared with the Departments of Health and Education to aid in planning for adequate service provision for high risk NICU graduates.

Research is an integral part of academic neonatal programs. An advantage of having a structured follow-up program is that it provides a mechanism to carry out research ranging from observational studies to randomized control trials, which include post-discharge outcomes. Currently, post-discharge neurodevelopmental impairment (NDI) is a common primary outcome for randomized control trials of NICU infants. NDI is defined as the presence of any of the following: moderate to severe CP, blind in both eyes, bilateral hearing impairment requiring amplification, and a Bayley mental developmental index or psychomotor developmental index score < 70. The use of NDI as a standard outcome for intervention trials evolved with the recognition of the frequent disconnect between perinatal outcomes and long-term outcomes. Examples include: 1) the aggressive administration of oxygen for relief of respiratory distress syndrome and subsequent retinopathy of prematurity/blind infants and 2) administration of postnatal steroids for weaning infants with broncho pulmonary dysplasia from a ventilator and subsequent CP [28–30]. The combined outcome of NDI or death

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

is often a choice for primary outcome since it is well recognized that death is a competing outcome with NDI. Utilization of the same definition for outcome among studies allows one to more readily compare the findings.

129.2 Methodological Issues and Follow-up Techniques

Differences in the reported incidence of neonatal morbidities (i.e., bronchopulmonary dysplasia [BPD]) or post-discharge outcomes (i.e., CP) may not be secondary to a specific intervention, but may be related to the specific characteristics of the center's NICU population (race, health insurance, poverty rates) and differences in management style [25, 31, 32]. Examples of medical management confounders include a center's policies for antenatal and obstetric care, variability of use of ventilators, medications, enteral and parenteral nutrition, and approaches to care for infants at the limits of viability. Neonatal factors affecting outcome include multiple versus singleton, inborn versus outborn, gestational age at birth and male versus female [33]. Social and environmental characteristics with significant heterogeneity include poverty level, public versus private insurance, level of parent education, single parent, English speaking versus bilingual or non-English speaking, cultural and religious differences, and availability of post-discharge medical, therapeutic, early intervention and educational services [34]. One of the challenges of follow-up studies which can contribute to bias in interpreting NDI rates is that poor and less educated families are less likely to return for follow-up.

129.2.1 Assessment in the Follow-up Program

Age of follow-up assessment impacts on the findings that can be reliably identified. In the first 2–3 years of life valid primary outcomes include moderate to severe CP, mental retardation, bilateral blindness, and bilateral hearing impairment. Current studies in the first 3 years often use the composite outcome of NDI as the primary outcome. At school age, however, a broad spectrum of subtle cognitive and neuropsychological deficits including impaired language skills, perceptual-motor skills, executive function, memory skills and attention problems become apparent and can be identified with standardized test administration.

The question that is often raised is at what age one can identify the real impact of a neonatal intervention. It is clear that neurologic status at the time of discharge has limited predictive value. There is consensus that major neurologic and sensory impairments can be identified at 18–24 months of age. Do the findings at 18–24 months predict school age findings? Longitudinal studies of preterm children have demonstrated variability in the progression of neurologic and developmental findings and include: stability [35], improvement [22], and worsening [36, 37] of neuropsychological findings. Limitations in the predictive value of early assessments may be secondary to differences in the follow-up rates, the inclusion or exclusion of children with major neurologic or sensory impairments, and the post-discharge environment. To minimize bias a follow-up rate of 90% is optimal. The validity of outcome data is always enhanced by utilization of a gender and age matched comparison group. Because of the exorbitant costs of tracking families to school age, the majority of trials and observational studies report outcomes at 18–24 months, an age at which major sequelae can be identified.

129.3 Assessments

One approach of outcome studies is to report comprehensive neurological, developmental, functional and health care status, which permits a total view of the child within the context of the family. Table 129.1 lists assessments suitable for follow-up studies of children up to 36 months of age divided into seven major categories: neurologic/neurosensory, cognitive, visual-motor/fine motor, speech and language, motor function/coordination, functional skills for daily living, and behavior.

129.3.1 Neurologic/Neurosensory

Because of its significant impact on the child and family, neurologic status, especially CP is an important quality indicator for a NICU. A diagnosis of CP is obtained by performing a systematic neurologic assessment [38]. CP is defined as a non-progressive central nervous system disorder with abnormal muscle tone in at least one extremity and abnormal control of movement and posture which interfere with age appropriate activities [38]. CP is a challenging diagnosis to make on infants less than a year of age because of the prevalence of hypertonicity among premature infants in the first year of life [39]. A definitive diagnosis of moderate to severe CP can be made by 18 months of age. CP is classified as moderate if the child can sit independently or with support, but an assistive device is required for ambulation, and severe if the child is unable to sit or walk even with support. Other neurologic findings identified in preterm children include hypertonicity, hypotonicity, seizures, dyspraxia, absent pincer, or facial palsy without a specific diagnosis of CP. Explicit criteria for describing hand function, sitting ability, and self-mobility have recently been developed [40]. Moderate to severe CP is a component of NDI.

Vision abnormalities are common among former preterm infants. The standard of care within tertiary care centers

Table 129.1 Assessments for neurodevelopmental and behavioral outcomes of VLBW infants

Tests		Ages
1. Neurologic and Sensory		
– Vision	Ophthalmologic examination	All ages
– Hearing	Auditory Brainstem Test	All ages
	Otoacoustic emissions	All ages
	Tympanometry	>6 months
	Vision reinforcement audiometry	>6 months
– Neurologic	Standard Neurologic Examination	All ages
categorize child as: Normal/Suspect/Abnormal		
Cerebral Palsy: Mild, Moderate, Severe		
2. Developmental/Intelligence and Developmental Tests		
– Bayley Scales of Infant Development II (BSID II) [42]		1–42 months
– Bayley III Scales of Infant and Toddler Development [160]		1–42 months
– Stanford Binet Intelligence Test, 4th edition (SB-4) [44]		2–18 years
– Differential Ability Scales [45]		2.5 to 18 years
– McCarthy Scales [46]		2.5 to 8.5 years
3. Vision Motor		
– Beery Developmental Test of Vision Motor Integration (VMI) [161]		Short form 3–8 years
4. Speech/Language/Vocabulary		
– Preschool Language Scale 3 [51]		Birth – 6 years
– Peabody Picture Vocabulary Test-Revised (PPVT-R) [162]		2 years 6 months – adult
– Early Language Milestone 2 (ELM2) [48]		Birth – 36 months
– Sequenced Inventory of Communicative Development-Revise (SICDR) [50]		4 mo – 4 years
5. Motor Function		
– Peabody Developmental Motor Scales [55]		0–7 years
– AGS Early Screening - Motor Profile [56]		2–11 years
– Palisano Gross Motor Classification Scale [67]		12 months – 12 years
6. Functional Status		
– WeeFim [62]		6 months – 8 years
– Vineland Adaptive Behavior Scale (VABS) [163]		Birth – 18 years 11 months
– Battelle Developmental Inventory (BDI) [164]		Birth – 8 years
– Pediatric Evaluation of Disability Inventory (PEDI) [74]		6 months – 7.5 years
7. Behavioral Outcomes		
– Child Behavior Checklist (CBCL 1.5-5) [165]		1.5 to 5 years

determines that VLBW infants have ophthalmologic examinations for retinopathy of prematurity prior to discharge with appropriate follow-up and intervention where appropriate. This has facilitated the identification of both severe and milder degrees of vision impairment. In addition, ongoing monitoring for the detection of strabismus and myopia are indicated. Bilateral blindness is included in the composite outcome of NDI.

Since NICU infants are at increased risk of all types of permanent hearing loss (HL) including neural (auditory neuropathy/auditory dyssynchrony) HL, the Joint Committee on Infant Hearing (JCIH) 2007 [41] has recommended automated auditory brainstem response (ABR) screening for all infants that require NICU care for > 5 days. Infants who fail the screen should have a comprehensive audiology assessment by 3 months of age. An audiology diagnostic assessment for infants less than 6 months at a minimum consists of a diagnostic auditory brainstem response (ABR) and otoacoustic emissions. Children who are at least 6 months of developmental age should also have a behavioral assessment, vision reinforcement audiometry, completed. Bilateral HL that requires amplification is included in the composite outcome of NDI.

129.3.2 Cognitive

The Bayley Scales of Infant Development (Bayley II) [42] has been the most commonly reported assessment of development from 4 months to 36 months of age and provides information for both cognitive (the mental developmental index [MDI]) and motor (the psychomotor developmental index [PDI]) domains. Bayley scores of 100 ± 15 represent the mean ± 1 standard deviation (SD) of a population of normal infants born at term. A score < 70 (2 SDs below the mean) is interpreted as evidence of developmental delay. The Bayley Scales of Infant Development III (BSID-III) is currently available for assessments from 1 month to 42 months of age [43]. In contrast to the Bayley II, the BSID-III consists of three domains: Cognitive, Language and Motor. In addition

there are subscores for receptive communication, expressive communication, fine motor and gross motor. The Bayley III was developed, in part, to separate cognitive from language domains to eliminate the bias imposed for children residing in a bilingual or non-English speaking household.

The Stanford Binet Intelligence Scale, 4th edition (SB-4) [44], the Differential Ability Scales [45] and the McCarthy Scales of Children's Abilities [46] are all tests of early cognitive/developmental ability. For studying populations of children, outcome data can be used to compare groups with one another (intervention *vs* control) or with normative data. Children's overall performance is often evaluated on a battery of tests which include visual perceptual skills [47], speech and language skills [48–52], cognition [44–46, 53, 54] fine and gross motor function [55–57] behavior [58–61] functional skills and health care status [62].

For an individual child early test results have limited predictive validity of cognitive status. First, tests administered at younger ages may not tap into the same psychological capacities as those present in older children, and the child resides within a changing environment with a spectrum of influences on development. School age outcomes have the most validity for final status of cognitive function.

At school age, cognitive functioning is assessed using a variety of different measures including the Stanford Binet Intelligence Scale – 4th edition, the Wechsler Preschool and Primary Scales of Intelligence – 3rd edition (WPPSI), the Wechsler Intelligence Scale for Children (WISC-III), the Woodcock-Johnson Psycho-Educational Battery – Revised, the Differential Abilities Scales, the McCarthy Scales of Children's Abilities, the British Abilities Scale, and the Kaufman Assessment Battery of Childhood. Each of these assessments provides an intelligence quotient (IQ) and subtest scores that allow for a limited assessment of specific areas of strengths and weaknesses. These tests, like the Bayley, have a mean of 100 with a standard deviation of 15 in the general population.

129.3.3 Visual Motor/Fine Motor Skills

The third category is visual motor and fine motor skills [47]. The most commonly used test for visual motor skills is the Beery-Buktenica Developmental Test of Visual-Motor Integration 4th edn (Beery VMI) [47]. The VMI assesses the degree to which visual-perception and motor behavior is integrated. It can help identify visual-motor problems before they develop into more serious difficulties. The VMI consists of geometric figures arranged in order of increasing difficulty. The child copies the figures as accurately as possible with pencil and paper. Fine motor skills can be assessed using the Peabody Developmental Motor Scales (PDMS) [55]. This test presents the child with a variety of fine motor tasks including block design, shape copying, cutting, folding, and manual dexterity tasks. A developmental motor quotient is calculated for each child's performance based on age. Mean developmental quotient is 100 ± 15.

129.3.4 Speech and Language

Speech language delays are common among preterms and a variety of tests are available. The Preschool Language Scale – 3rd edition (PLS-3) [51] is a standardized assessment which includes two subscales-auditory comprehension and expressive communication – to assess attention, vocal development, social communication, semantics, language structure, and integrative thinking skills. The Peabody Picture Vocabulary Test (PPVT-R) [49] is a non-verbal, multiple-choice test that measures receptive vocabulary. The Early Language Milestone Scale, 2nd edn (ELM Scale-2) [48] assesses speech and language development in three areas: Auditory Expressive, Auditory Receptive, and Visual. The Sequenced Inventory of Communication Development Revised edition (SICDR) [50] is a diagnostic test that evaluates the communication abilities of children who are between 4 months and 4 years of age. It has been used successfully with children with sensory impairments and varying degrees of retardation [63–66].

129.3.5 Motor Function/Coordination

The PDMS [55] are a test of gross and fine motor skills of children aged birth through 83 months. The Early Screening Profiles (ESP) [56] measures motor, cognitive/language, and developmental skills in children. The ESP consists of three profiles: Motor Profile, Cognitive/Language Profile, and Self-Help/Social Profile. A Gross Motor Function Classification System (GMFCS) skill level derived from the work of Russell et al and Palisano et al [67–69] is used in outcome studies to assess gross motor function. A scoring system consisting of skill levels ranging from 0 to 5 was developed by Palisano and colleagues [67–69]. This system was developed as a method for assessment of a child's motor function by direct observation of the child's gross motor performance. It describes a child's function, not the fluidity of their movements. Palisano's system classifies gross motor function on a 5 point scale. Normal function at 18–24 months is defined as Level 0 and involves the ability to walk at least 10 steps independently. An infant at Level 1 can sit with hands free, creep or crawl on hands and knees, pull to stand and cruise or walk with hands held. Those at Level 2 use their hands for sitting support, creep on their stomach and may pull to stand; at Level 3 require external support to sit, roll and may creep; at Level 4 maintain head control in a supported sitting position and can roll prone to supine. Level 5 is the inability to maintain antigravity movements of the head and trunk [67, 70]. The GMFCS does not assess fine motor skills.

129.3.6 Functional Measures

The sixth area of assessment includes functional measures of daily living skills. Functional assessment is the process of determining as accurately as possible an individual's ability to perform the tasks of daily living and to fulfill the social roles expected of a physically and emotionally healthy person of the same age and culture [71]. In children, the key tasks include feeding, dressing, bathing, maintaining consistency, mobility, communication, play, and social interaction [72]. The social roles expected include involvement with peers and attending school. Four functional outcome measures are currently available [62, 73, 74]. The Pediatric Evaluation of Disability Inventory (PEDI) assesses developmental skills in self-care, mobility, and social function, care giver assistance and modification of environment in children 6 months to 7.5 years [74]. The Vineland Adaptive Behavior Scale (VABS) measures communication, daily living, socialization, and motor skills in children birth to 18 years. The Vineland has been used with children with motor, cognitive, and sensory disabilities. Rosenbaum et al have advocated its use for describing degrees of disability in children who were very low birth weight [75]. The Battelle Developmental Inventory (BDI) is a developmental educational assessment battery for children age 0–8 years with or without developmental delays. The Battelle consists of five domains: personal-social, adaptive, motor, communicative and cognitive. It is widely used in early intervention. The Functional Independence Measure for Children (WeeFIM) [62] can be used in children with and without disabilities through age 8 years. After age 8 years, individuals with developmental skills less than 8 years can continue to be assessed with the WeeFIM [76] through adolescence.

129.3.7 Behavior and Psychology

Evaluations of behavior are routinely obtained in infancy and childhood by parent, teacher, or subject interviews with standardized measures of behavior, attention, adaptive skills and depression. The Child Behaviour Checklist [77] is a parent-report questionnaire designed to describe social competencies and emotional/behavioral issues of children and is commonly used in follow-up studies. It has a version for 1½ to 5 year olds and a version for ages 4–18. It provides scores for internalizing (i.e., anxious, depressive, and overcontrolled) and externalizing (i.e., aggressive, hyperactive, noncompliant, and under-controlled) behaviors, and has scores that were derived for withdrawn, somatic complaints, anxious/depressed, social problems, thought problems, attention problems, delinquent behavior, aggressive behavior, and the presence of any behavior problem. The Conners Rating Scales [78] are questionnaires designed for parents or teachers to describe symptoms of inattention, hyperactivity, and oppositionality in school age children. Multiple different measures of childhood depression exist and have been studied in this population. The Modified Checklist for Autism in Toddlers (M-CHAT) [79, 80] and the Pervasive Developmental Disorders Screening Test II (PDDST II) [81] are currently available as screeners for autism spectrum disorder.

129.4 Definitions of Normal and Abnormal Outcome

Although the traditional outcome described for infants is an abnormal outcome defined as NDI, more recent investigators have attempted to define a normal outcome. A recent study by Gargus et al [82] defined a normal outcome at 18–22 months corrected age as infants who were unimpaired; they had both Bayley-II [42] MDI and PDI scores within 1 standard deviation of the mean, normal neurological findings, normal vision, hearing, normal functional ability for swallowing by parent report, and normal ambulation. In addition, infants were classified as having mild impairments if they had any of the following: Bayley MDI or PDI between 70 and 84, CP, mild other neurological findings, or minor sensory impairments (glasses, transient conductive hearing loss, unilateral hearing loss, or unilateral blindness). Severe NDI was defined as any of the following: Bayley MDI < 70, Bayley PDI < 70, moderate to severe CP, bilateral blindness or bilateral hearing loss requiring amplification. The last definition is the traditional NDI.

129.5 VLBW Outcomes

The majority of published reports of neurodevelopmental outcome in infancy focus primarily on the incidence of moderate to severe disability [83]. This has been the neurodevelopmental outcome of interest due to the severity of the developmental impact of these severe and often combined morbidities. Unlike mortality rates which have dramatically improved [1, 6, 8, 83–87], the incidence of moderate to severe disabilities has not changed significantly over the past 20 years [1, 8, 83–91]. Rates are highest in ELBW populations, and like mortality rates, rates of disability generally increase with decreasing gestational age and birth weight [83, 87, 90, 92–94]. Worldwide rates of severe disability in infants of 24–25 weeks' gestation range from 22 to 45% in infants born at 24 weeks, 12 to 35% in infants born at 25 weeks, and 9 to 37% in infants born < 800 grams birthweight [83, 88].

In the NICHD Neonatal Research Network, rates of NDI in the 1990s ranged from 28 to 40% in infants born 27–32 weeks and 45 to 50% in infants born 22–26 weeks [90]. Only 21% of all ELBW infants were unimpaired (no CP, normal

cognitive and motor scores, no visual or hearing impairment) at 18 months [8]. Regional and local studies in the 1990s report similar wide ranges of major NDI rates, from 20 to 48% [1, 84, 85, 87, 91, 93, 95].

129.5.1 Neurologic/Neurosensory

Extremely preterm infants are born during a period of active brain development and maturation, placing them at extremely high risk for brain injury from hypoxia, ischemia, undernutrition, and infection, which are associated with both intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL). PVL is injury to the periventricular white matter as a result of hypoperfusion and infarction. It is visualized radiographically as echolucency, echodensity, or cystic degeneration. Although IVH, ventriculomegaly at term and cystic PVL are all associated with CP, cystic PVL is the strongest predictor [70].

Rates of CP in ELBW vary from 5 to 30% [8, 20, 70, 83–85, 87–94, 96–101] but are most commonly sited at 15 to 23% [8, 70, 90, 93, 94, 98–100]. The most common form of CP in this population is spastic diplegia, accounting for 40 to 50% of all cases, followed by spastic quadriplegia, and hemiplegia [70, 93, 100]. This is not surprising as PVL lesions involve injury to the white matter that contains the descending motor tracts for the lower extremities. More extensive lesions also involve upper extremity motor tracts.

While CP is the most well known and potentially most disabling motor abnormality associated with prematurity, infants born preterm often demonstrate less severe differences in their neurologic development. During the first year of life transient dystonia is a common deviation in the motor development of VLBW infants [39, 102, 103]. Transient dystonia was first described in 1972 by Drillien as transient abnormalities on neurologic examination in close to half of all low birth weight infants (< 2000 grams) in the first year of life. More recently these transient findings have been re-described as occurring in 21 to 36% of preterm infants with a peak incidence at 7 months corrected age [102–104]. The motor features described include increased extensor tone of the trunk and lower extremities and increased adductor tone in the lower extremities leading to shoulder retraction and hip rotation, persistent primitive reflexes, head lag on pull to sit and delayed supportive responses [39]. The presence of findings consistent with dystonia increases the risk of later cognitive and motor problems including CP but have a low specificity as they are transient in 80% of the infants in which they occur, disappearing gradually between 8 and 12 months of age. The other 20% often go on to be diagnosed with CP.

While much less common than motor disabilities, rates of neurosensory disabilities are higher in ELBW infants than the general population. Unilateral or bilateral blindness occurs in 1–10% of ELBW infants [8, 20, 83–85, 87–89, 91, 93, 94, 97, 100, 105]. Milder visual impairments including myopia, strabismus, and lack of stereopsis (depth perception) occur at rates of 9–25% [92, 94, 97, 100, 106].

Hearing impairment requiring amplification is reported in 1–9% of ELBW infants [8, 20, 83, 84, 87, 89–94, 97, 100]. Milder hearing impairment has been reported in 11–13% [94, 100]. When transient conductive or unilateral hearing loss is included, rates of milder impairment are as high as 28% [97]. These rates of neurosensory impairment persist at school age [98, 99, 107, 108].

129.5.2 Cognitive

The most common severe impairment seen in VLBW and ELBW infants at 18 and 30 months is cognitive impairment, defined as scores that are more than 2 standard deviations below the mean on standardized cognitive testing. Most published follow-up studies of ELBW infants to date have used the Bayley Scales of Infant Development II (Bayley II) as the measure of cognitive functioning between 6 months to 3 years [42]. Average score for ELBW infants at 18–22 months corrected age on the Bayley II in the NICHD is 76 [94] but varies from center to center with a range of 70 to 83 [97]. Center and regional reports site higher average MDIs. In a cohort of <1000 gram infants born from 1982 to 2002, Wilson-Costello and Hack report average MDIs of 84–86 (and 83–89 in a subset of < 750 gram infants) at 20 month follow-up [84, 87, 91]. Wood et al reported similar results at 30 months corrected age in a cohort of 20–25 week infants in the United Kingdom whose average MDI was 84 [100].

Like rates of NDI and CP, rates of cognitive impairment, defined as a score < 70 on cognitive assessment, vary worldwide, and are inversely proportional to gestational age and birth weight. Rates of cognitive impairment range from 14 to 39% at 24 weeks, 10 to 30% at 25 weeks [83], 4 to 24% at < 26 weeks, and 11% to 18% at < 29 weeks worldwide [89, 92]. In infants born < 800 grams, rates of cognitive impairment range from 13 to 50% [20, 83, 85, 88, 89] and at < 1250 grams 26% [89]. Rates of cognitive impairment are reported at 37–47% in 22–26 week infants [8, 90] 23–30% in 27–32 week infants [90] and 34–37% in all infants < 1000 grams in the NICHD [94, 109]. Wilson-Costello and Hack site 20–26% rates of cognitive impairment in their cohort of ELBW infants at 18 months [84, 87, 91] and at 30 months, 30% of Wood's cohort had cognitive impairment [100].

While cognitive functioning can by measured in infancy, it may not be predictive of cognitive functioning later in life. The assessment of an infant's cognitive function is highly dependent on their motor, language, and social-emotional development. Hack et al found that MDI at 20 months corrected age was not predictive of cognitive functioning at 8 years of age. In their cohort of 330 ELBW infants, mean MDI at 20 months was 76 compared to a mean cognitive score of 88 at 8 years, and rate of cognitive impairment dropped from 39% at 20 months to 16% at 8 years. The positive predictive value

Table 129.2 Cognitive outcomes at 12–36 months corrected age for ELBW infants

Year of birth	Sample size	BW/GA	Subgroups	Group (Mean MDI)	Group (MDI <70)	Age months	Location	Author
1982–1992	88	<750	1982–88 1990–92	1982–88 (83) 1990–92 (89)	1982–88 (26%) 1990–92 (20%)	20 m	Local center, USA	Hack et al [84]
1993–1999	246	<750	N/A	N/A	46%	18–22 m	NICHD, USA	Shankaran et al [20]
1987–1995	Review	<800	N/A	N/A	13–48%	12–36 m	Worldwide review	Hack et al [83]
1977–1994	Review	<800 <27 w	N/A	N/A	14% (0–50%)	Variable	Worldwide review	Lorenz et al [88]
1979–1994	216	<800	1979–84 1984–89 1989–94	1979–84 (96) 1984–89 (96) 1989–94 (93)	1979–84 (13%) 1984–89 (20%) 1989–94 (13%)	12 m	Regional, USA	O'Shea et al [85]
1982–1998	623	<1000	1982–89 1990–98 500–749 g 750–999 g	1982–89 (86) 500–749 g (81) 750–999 g (88) 1990-98 (84) 500–749 g (80) 750–999 g (85)	1982–89 (20%) 500–749 g (28%) 750–999 g (18%) 1990–98 (26%) 500–749 g (34%) 750–999 g (22%)	20 m	Local center, USA	Wilson-Costello et al [87]
1982-2002	872	<1000	1982–89 1990–99 2000–02	1982–89 (86) 1990–99 (84) 2000-02 (86)	1982–89 (20%) 1990–99 (24%) 2000–02 (21%)	20 m	Local center, USA	Wilson-Costello et al [91]
1992–1995	221	<1000	N/A	75	42%	20 m	Local center, USA	Hack et al [93]
1993–1994	1151	<1000	<500 g 501–600 g 601–700 g 701–800 g 801–900 g 901–1000 g	<500 g (78) 501–600 g (73) 601–700 g (74) 701–800 g (74) 801–900 g (77) 901–1000g (79)	<500 g (31%) 501–600 g (45%) 601–700 g (41%) 701–800 g (42%) 801–900 g (35%) 901–1000 g (31%)	18–22 m	NICHD, USA	Vohr et al [94]
1993–1994	1151	<1000	12 centers	70–83	17–62%	18–22m	NICHD, USA	Vohr et al [97]
1993–1999	777	<25 w ≤23 w 24 w	1993–96 1996–99	1993–96 (75) 1996–99 (72) 1996–99 (47%) ≤23 w (52%) 24 w (44%)	1993–96 (40%) ≤23 w (38%) 24 w (40%)	18–22 m	NICHD, USA	Hintz et al [8]
1995	251	≤25	≤23 w 24 w 25 w	84	MDI <70 (30%) ≤23 w (27%) 24 w (30%) 25 w (30%)	30 m	UK and Ireland	Wood et al [100]
1993–1998	3785	22–32 w	1993–94 1995–96 1997–98 22–26 w 27–32 w		1993–94 22–26 w (42%) 27–32 w (30%) 1995–96 22–26 w (39%) 27–32 w (26%) 1997–98 22–26 w (37%) 27–32 w (23%)	18 m	NICHD, USA	Vohr et al [90]

of having a low cognitive score at 8 years (< 70) given a low cognitive score at 20 months (< 70) was only 0.37 [108]. Ment et al reported recovery of test scores in a cohort of VLBW infant [110]. Mean expressive language scores increased from 88 at 3 years to 99 at 8 years of age and full scale IQ increased from 90 to 96.

Mean IQ for VLBW and ELBW infants at school age (5 to 14 years) ranges from 82 to 105 [99, 108, 110–117].

Although the mean IQ is within the average or low average range for children born ELBW or VLBW, they have significantly lower IQ scores than their normal birth weight peers (0.5 to 1.0 SD lower) [98, 111–118] and significantly higher rates of cognitive impairment [98, 99, 119]. Cognitive scores at school age remain significantly correlated with gestational age and birth weight [37, 111, 112, 116]. While environmental factors such as type of health insurance, bilingual household, income level, single parent, teen-age mother and level of maternal education are known to impact on intelligence, differences in IQ between preterm and term controls persist after adjustment for these confounders [120].

In addition to impairments in global cognitive functioning, more subtle cognitive impairments are often detected in school age. These higher prevalence, lower severity dysfunctions reportedly occur in 50–70% of children born VLBW [95]. Children born VLBW or ELBW have relative impairments of executive functioning [116, 121–123], visual-motor skills [123], memory [116, 122], especially verbal memory [124]. They score lower on tests of academic achievement [108, 113, 122], perceptual-organizational skills [116, 118], visual processing tasks [116, 118] and adaptive functioning [116, 122] compared to their normal birth weight peers. Even ELBW infants without neurosensory or cognitive impairment have higher rates of learning disabilities [111, 117] especially in math [115, 116, 118, 125], ranging from 25 to 40% [111, 118].

So it is not surprising that ELBW infants have higher rates of academic underachievement and need for special education services [111, 114, 119]. Like their cognitive scores, mean scores on formal tests of academic achievement fall within the normal range (94–105), but are lower than scores of normal birth weight peers [111, 114]. Teachers of VLBW infants report rates of below average school performance in all academic areas, ranging from 24 to 41% [111, 113, 119, 126]. Approximately 25% of VLBW infants and up to 62% of ELBW infants receive special education services [107, 114, 116, 119, 127, 128]. Between 15 and 34% required grade repetition [115, 116, 128, 129].

An increasing number of investigators have reported on cognitive and academic abilities of former VLBW and ELBW teenagers and young adults [37, 99, 116, 122–124, 128, 130]. ELBW teens continue to have mean cognitive scores in the average to low average range but persist in having significantly lower cognitive and academic scores than teens born normal birth weight [127, 131, 132] and significantly higher rates of cognitive impairment [127, 132]. Cognitive differences are greatest in areas of visual-perceptual tasks [131]. Academic differences are seen in reading and mathematics [131]. As a result, only 56–74% of preterm children, significantly fewer than normal birth weight teens, graduate from high school [127, 132]. Hack's report on a single center cohort of VLBW infants showed significant gender differences in graduation rates: 66% of VLBW males compared to 75% for term males and 81% for VLBW females compared to 90% for term females [132].

129.5.3 Visual Motor/Fine Motor

Difficulties with fine motor skills and with visual-motor skills, described as problems with visual perception, visual motor control, hand-eye coordination, or visual-motor integration, are common in children born VLBW or ELBW. These difficulties can impact on academic performance and functional abilities. Fine motor difficulties have been described in as many as 70% and fine motor difficulties resulting in impairment in 23% at 5 years of age [133]. Average scores on the Beery VMI are significantly lower in ELBW infants than term controls at age 7–8 (91 *vs* 97, $p < 0.001$) [134] and age 12 (82 *vs* 92) [135] and visual motor integration deficits (VMI < 70) are found in 16–24% of ELBW infants at school age [134, 135].

129.5.4 Speech and Language

Speech and language develop atypically during early childhood in previously ELBW infants, with delays in the acquisition of expressive language, receptive language, and articulation [136–138]. Children born at or before 25 weeks gestation have significantly lower total scores on the PLS (90 *vs* 104) and significantly higher rates of language impairment (16 *vs* 2%) at 6 years of age. Scores on auditory comprehension, expressive communication and articulation subtests of the PLS are uniformly lower [139]. In addition, rates of language impairment, defined as standard scores of < 70 on language assessment, are higher. Former ELBW infants have significantly lower scores (92 *vs* 105) and higher rates of impairment (13 *vs* 4%) on the PPVT, as well as lower expressive, receptive, and total scores (85–87 *vs* 100–103) and higher rates of impairment (22–24% *vs* 3–4%) on Clinical Evaluation of Language Fundamentals (CELF) at age 12 [135]. While language development in infancy and early childhood is often an early proxy for overall cognitive development, specific language deficits have been described including phonological short-term memory [140] and prosodic processing [141]. At 12 years of age, children who are born <1250 grams have less pronounced differences on tests of lower level language skills (phonological processing, phonemic decoding and sight word reading) compared to term controls, but exhibit significantly more difficulty with higher level skills (syntax, semantics, verbal language memory and reading comprehension) [135]. In addition, when given a semantic processing task, preterm children have abnormal patterns of brain activity on functional MRI, resembling the brain activity of term controls during phonologic processing [66].

129.5.5 Motor Function/Coordination

Arguably more important than the type or location of impairment is the functional level of the affected infant. Level of

gross motor function is most commonly assessed and categorized using Palisano's GMFCS [67]. In an NICHD Neonatal Network study, though 27% of a cohort of ELBW infants diagnosed with CP at 18–22 months had moderate to severe gross motor function (Level 3–5), 28% had gross motor function consistent with level 0 or 1 and were ambulatory [70]. It is important to remember that a diagnosis of CP includes a wide spectrum of motor performance.

More subtle motor abnormalities are also seen more commonly in former preterm infants and children born preterm are more likely to have difficulty with motor coordination. In the past these children were often labeled as clumsy but in recent years the diagnosis of developmental coordination disorder (DCD) has been used. DCD is defined as an impairment in motor performance sufficient to produce functional impairment that cannot be otherwise explained by the child's age, cognitive ability, or neurologic or psychiatric diagnosis. Scores of less than the 5th to 10th percentile on the Movement Assessment Battery for Children (MABC) are considered diagnostic. DCD is found in 31–34% of VLBW and 50% of ELBW infants [134, 142].

129.6 Functional Outcomes

As a result of the high rates of associated cognitive, motor, neurosensory, and behavioral difficulties seen in children who were born preterm, these children have higher rates of functional limitations than children who were born at term [107]. These functional delays are observed in VLBW infants with and without severe impairments. While 93% of ELBW infants achieve sitting balance, 83% walk, and 86% feed themselves independently by 18–22 months corrected age, more subtle functional deficits become apparent later in life [94]. At 10–14 years of age, 27% of children who were VLBW and 32% of those who were ELBW report restricted physical activity; and 24% of VLBW and 29% of ELBW report they are unable to participate in sports [107]. Functional outcomes are considered particularly important by parents.

129.7 Behavioral and Psychological

Very low birth weight has been associated with a wide variety of behavioral and psychological diagnoses and disabilities. Recent concern has arisen that rates of Autism Spectrum Disorder (ASD) may be higher in ELBW infants than previously thought. Though low birth weight (< 2500 grams) may result in a 2–3 fold increase in the risk of ASD [143, 144], true risk of ASD in very preterm infants is unknown. Two prior studies have investigated rates of autistic characteristics in children born very low birth weight (VLBW, <1500 grams). Indredavik et al [145] demonstrated a trend toward higher scores on the Autism Spectrum Screening Questionnaire in a population of 56 VLBWs at 14 years of age compared to full-term controls. Limperopoulos et al [146] recently reported 25% of VLBW infants screen positive on the M-CHAT at 18 months. However the M-CHAT was developed for use in the general population and not for a high risk population such as VLBW infants where false positive screening rates are likely high due to associated cognitive, language and motor delays. In addition, no diagnostic confirmation was performed [146]. Further studies are needed to determine the true risk of autism in this population.

At school age (8–12 years old), parents and teachers of VLBW/ELBW infants report higher rates of inattention and hyperactivity [111, 112, 116, 117, 119, 129, 145, 147, 148] with rates of 23–27% in VLBW and 33–37% in ELBW infants [117, 119, 145, 147]. At 12–14 years of age one quarter to one half of VLBW/ELBW infants have symptoms of anxiety and/or social withdrawal [117, 119] 8–14% meet criteria for generalized anxiety disorder (compared to 1–4% of peers) [145, 147] and 25–28% meet criteria for a psychiatric disorder (compared to 7–10% of peers) [145, 147]. At 17 and 20 years of age, ELBWs score higher on measures of inattention, anxiety/depression, withdrawn behavior, and social problems at 17 years of age [131, 149]. In addition, VLBW teens score significantly lower on measures of self esteem [115, 131]. They report less confidence in their athletic, school, romantic, and job related abilities [131]. In contrast, VLBW adults report lower rates of alcohol and drug use, sexual activity, and pregnancy than adults born normal birth weight [132, 149].

129.8 Late Preterm

While the majority of neonatal outcomes research has focused on the ELBW infant, more recent studies have brought a long neglected population of infants to our attention, the late preterm population. During the 1990s the rates of delivery at 40 or more weeks gestation decreased while rates of deliveries between 34 and 36 weeks increased steadily [150]. From 1990 to 2005 the rate of late preterm births increased from 7.3 to 9.1% of all births [151]. Compared to term infants, these late preterm infants have higher mortality rates [152–154]. They also have higher rates of neonatal morbidities such as respiratory distress, temperature instability, hypoglycemia, kernicteris, apnea seizures, infection, and feeding problems [151–153, 155, 156]. All of these morbidities have the potential to increase the risk of long-term neurodevelopmental sequelae. In addition, the brain of the late preterm infant is more immature than the term infants' brain. At 34 weeks there are significantly fewer gyri and sulci, and the brain weighs an estimated 60% of that of a term infant [156]. While there is a large body of literature that addresses the neurodevelopmental outcome of VLBW and ELBW infants, there is a paucity of information published about the neurodevelopmental sequelae

of late preterm birth. Infants born 34–36 weeks are 3.39 times as likely as term infants to develop CP and 1.25 times as likely to have cognitive impairment [157]. They are more likely to qualify for special needs preschool and are more likely to have problems with school readiness [158]. In kindergarden and first grade they have lower reading scores, teachers report math skills below those of their full-term peers, and they are more likely to qualify for special education services [159].

129.9 Summary

In summary, preterm infant survivors remain at increased risk of adverse neurodevelopmental outcomes. Longitudinal assessment of outcomes is indicated to identify neurosensory, developmental, and behavioral sequelae and to ensure appropriate supports and interventions are provided. Continued randomized trials are needed to identify perinatal interventions that optimize outcomes.

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130

Neurological Examination of the Newborn Infant

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130.1 Aims of the Neurological Examination and Basic Requirements

A simple neurological screening examination should be performed on all newborn infants as part of the general medical examination. It should consist of an assessment of state of consciousness-reactivity, spontaneous motor activity, neck, trunk and limb muscle tone and some primitive reflexes (e.g. suck and grasp. Not all experts include the Moro reflex in the general examination on the basis that it is cruel to startle a person) [1].

Several schemes of neurological examinations have been developed during the last decades to document clinical neurological signs and their evolution or their disappearance [2–5]. The number and type of clinical neurological signs included in the various schemes are very heterogeneous. Some are comprehensive examinations designed essentially for research purposes, others are simpler clinical tools that aim to assess the integrity of the central nervous system (CNS).

Along with the definition of neurological signs and their evolution, the neurological examination tries to answer other questions such as the recognition of age appropriate development of the CNS, the presence of an acute or a chronic brain disorder, the central or peripheral nature of the problem and the prognosis for that infant. These questions cannot be all answered by the same protocol as each has its own peculiarities and strengths.

Preterm infants after birth and small for gestational age infants demand a neurological examination which includes a number of items, assessing the age appropriateness of neurological development (CNS maturity) throughout the preterm period and at term [4, 7]. The recognition of an acute neonatal encephalopathy in full-term (or near-term preterm) infants requires an examination specifically designed for acutely ill babies. The recognition of emotional and temperamental characteristics, as well as the response to stressors, requires the use of behaviourally oriented protocols [3, 6].

The choice of neurological examination depends on the study group and the purpose of the examination. According to Prechtl, there are a few basic requirements for the neurological assessment and for its successful use in clinical practise [8]. It should include items related to the age-specific repertoire of the CNS, which changes very rapidly during the pre-and early postnatal period.

The diagnostic procedure must be non-invasive and relatively quick to perform, as the neurological examination should be included within the routine clinical assessment and should be repeated to document the evolution of neurological abnormalities and to facilitate prognostic accuracy. Reliability and prognostic capability are two critical requirements. There should also be good inter-observer agreement should also apply.

This chapter describes the different approaches to the neurological examination of the newborn infant and neurological protocols that best fulfil the above criteria.

130.2 Available Neurological Examinations

Because each method represented an improved approach to understanding the neonatal nervous system, the methods will be considered chronologically. Few studies have compared the different protocols and it cannot be claimed that one is better than another. The French school (André Thomas and Saint-Anne Dargassies, Amiel-Tison) highlighted spontaneous posture, some neonatal reflexes and various aspects of muscle tone. Active tone considers “fonctions de redressement” and is associated with spontaneous movements. Passive tone is the capacity of muscle to be lengthened when the joints are moved passively and the tone that can be seen by allowing the extremity to swing at each joint. Saint-Anne

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Dargassies described the ontogenesis of muscle tone in premature infants: outlining the progressive maturation of muscle tone, with development of flexor predominance in caudal-cephalic direction [2, 9].

The Amiel-Tison neurologic assessment at term (ATNAT) has recently been updated for clinical application. The complete procedure takes a few minutes. A simple 0, 1, and 2 scoring system is proposed. The ATNAT is part of a set of three different instruments based on a neuro-maturative framework. By sharing a similar methodology and scoring system, the use of these three assessments allows for the follow-up of high risk children from 32 weeks post-conception to 6 years of age [2, 10].

Prechtl and co-workers [10, 11] developed a comprehensive examination, based on the assessment of spontaneous and elicited responses. They studied the various neurological responses of normal healthy term infants and documented any abnormality in the responses. They analysed and documented trunk and limb posture, spontaneous movements of the various parts of the body and of the eyes, the power and the resistance to passive movements, invluntary activities such as skin color and respiration, skin reflexes (abdominal, cremasteric, anal reflex), osteo-tendon reflexes, evoked responses such as palmar and plantar reflex, Babinski, Moro, tonic neck asymmetric reflex, rooting, sucking, placing, Bauer reflex, crawling, automatic stepping, abnormal movements (athetoid, tremors) and crying. Each single response and behavior was evaluated in its optimal state. They demonstrated the dependency of these patterns on an infant's behavioral state. When the behavioral state was considered, normal infants responded consistently to the elicited stimuli. Non-responsiveness, despite being in the right behavioral state, was considered an abnormal neurological sign [12–14].

130.2.1 Behavioral State

Recognition of behavioral states was a major advance in the field of neonatal neurology. Behavioral states are an expression of the complexity of the nervous system; they are relatively stable over time (ranging from a few minutes to half an hour or more) and occur in particular sequential patterns. They are recognized on the basis of three to four essential physiological parameters (respiration, eyes open or closed, eye movements, body movements) that remain stable over time for at least a few minutes. Prechtl identified two states of sleep (state 1 or quiet sleep, state 2 or active sleep) and three states of wakefulness (state 3 or quiet wakefulness, state 4 or active wakefulness, state 5 or crying) [12]. Evoked reflexes and responses as well as spontaneous activity (such as body and eye movement and respiratory patterns) change according to the behavioral states.

The Groningen school showed that two sleep states are recognizable from the 36th week of gestation, while before 34 weeks' gestation, indeterminate sleep is predominant. Before 28 weeks, the newborn states of wakefulness and sleep are difficult to distinguish [12].

The recognition of different behavioral states is fundamental for the diagnosis of neurological integrity in the newborn. One of the earliest and most sensitive sign of cerebral dysfunction is disorganization of the behavioral state. Such disorganization of state can be a loose state cycle, a qualitative alteration of a single state or a physiological parameter defining states or the presence of an unstable sleep pattern [12, 13].

A useful and major innovation by Prechtl was the clustering of neurological signs into diagnostic syndromes. Using the computerized analysis of individual neurological deviations, he recognized four main syndromes: the hyper-excitable syndrome with increased responsiveness to various stimuli, the apathetic syndrome with decreased responsiveness, the hemi-syndrome with asymmetry of response, and the comatose syndrome [13, 14].

Upto this point, the Prechtl examination was the most detailed and comprehensive examination: it gave reliable information about the newborn infant neurological status and was able to differentiate central from peripheral involvement. The shortcomings of the Prechtl examination are the long time to perform the examination (about 30 minutes) and its complexity: a fairly large number of items are explored and each has to be observed in its optimal state. Furthermore it is restricted to the full-term infant in good physical health, because the degree of handling required may be unsuitable for the ill and fragile baby [8].

Prechtl also developed a shorter version of the protocol, suitable for neurological screening. It includes evaluation of head, trunk and limb posture, eye coordination, normal and abnormal limb movements, head, trunk and limb resistance to passive movement, traction test and the Moro response. The neurological screening examination cannot replace a full neurological examination: it gives more false positive results and, if abnormalities are suspected, a full examination is required. The short version of Prechtl examination is illustrated in Fig 130.1.

Brazelton [3] developed the Neonatal Behavioural Assessment Scale (NBAS), a structured scheme for term infants which combined 27 items of behavior and 20 reflex items derived from the neurological examination by Prechtl. The behavioral and neurological items can be grouped into seven major clusters of items (Fig. 130.2). The NBAS was designed to score the babies' behavioral repertoire and to document the organized responses to various environmental events. It was not originally conceived as a neurological examination but as a tool to evaluate the behavioral characteristics of the single baby. The intention was to predict temperament and emotional control, allowing the parents to perceive the baby as a person from the very first. The author suggested performing the test in front of the mother who would then recognize how bright and talented her baby was. In this context the Brazelton scale aims to reinforce early

Name ..... Date of birth ..... Gestational age .....

Date of examination ..... Gestational age at examination .....

Behavioral state:

Posture (head in the midline)			
Superior limbs	Semi-flexion	Flexion	Extension
Inferior limbs	Semi-flexion	Flexion	Extension
Symmetrical posture	Yes		No
Opisthotonus	Yes		No
Frog posture	Yes		No
Head turned to one side	Yes		No

Eyes		
In the middle	Yes	No
Constant deviation to one side	Yes	No
Constant strabismus	Yes	No

Spontaneous movements		
Arm and leg alternative movements	Yes	No
Symmetrical movements	Yes	No
Normal strength and length movements	Yes	No
Tremor	Yes	No
Exaggerated fast movements	Yes	No
Startles	Yes	No
Seizures	Yes	No

Resistance to passive mobilisation (passive tone)			
Neck	Scarce	Normal	High
Trunk	Scarce	Normal	High
Arms	Scarce	Normal	High
Arms	Symmetrical	Asymmetrical	Right/Left
Legs	Scarce	Normal	High
Legs	Symmetrical	Asymmetrical	Right/Left

Traction test			
Arm resistance	Scarce	Normal	High
Head control	Less than 3 sec	3-10 sec	More than 10 sec
Sucking	Absent	Weak	Present and strong

Moro reflex		
Abduction and extensions	Yes	No
Flexion absent, only abduction	Yes	No
Asymmetrical	Yes	No
Tremor	Yes	No

Diagnosis	**Normal**	**Suspect**	**Abnormal**

Fig. 130.1 Prechtl screening neurological examination

Name ..... Date of birth ..... Gestational Age .....
Weight ..... Length ..... Head circumference .....
Date ..... Examiner .....

INFANT BEHAVIOUR										
HABITUATION	9	8	7	6	5	4	3	2	1	Comment
Response Dec.-Light										
Response Dec.-Rattle										
Response Dec.-Bell										
Response Dec.-Foot										
SOCIAL-INTERACTIVE	9	8	7	6	5	4	3	2	1	
Animate Visual										
Animate Visual+Auditory										
Inanimate Visual										
Inanimate Visual+Auditory										
Animate Auditory										
Inanimate Auditory										
Alertness										
MOTOR SYSTEM	9	8	7	6	5	4	3	2	1	
General Tone										
Motor Maturity										
Pull to sit										
Defensive										
Activity Level										
STATE ORGANIZATION	9	8	7	6	5	4	3	2	1	
Peak of Excitement										
Rapidity of Build-up										
Irritability										
Lability of States										
STATE REGULATION	9	8	7	6	5	4	3	2	1	
Cuddliness										
Consolability										
Self-Quieting										
Hand to Mouth										
AUTONOMIC SYSTEM	9	8	7	6	5	4	3	2	1	
Tremoulousness										
Startles										
Lability of Skin Color										
SMILES										

Fig. 130.2 NBAS scoring form

mother infant interaction. It has also the power to evaluate cultural and ethnic differences in neonatal behavior in different settings. Despite this premise, the examination has often been used as a neurological evaluation. The Brazelton test had a great impact on pediatricians: its major contribution was towards abandoning the idea of the newborn infant as incompetent, immature and disorganized. Attention was drawn to the amazing capacities of the newborn infant for attention, with very predictable behaviors and the capacity to respond and interact with the environment. Attention was drawn to the baby's sensory capacities and visual and auditory responses: visual orientation to an inanimate stimulus (a red ball) and to the human face, auditory orientation to the sound of a rattle, bell and the human voice were introduced as integral part of the assessment. Some of these items were later adopted in other neurological schemes [3].

Based on the NBAS of Brazelton's assessment of term infant, Als et al [15] developed the Assessment of Preterm Infant Behaviour (APIB). This is a strategy to document systematically behavioral ingredients of the premature infant, from the stage when the baby can first be handled in room temperature and room air, without technological aids, to the stage when

Hammersmith Neonatal Neurological Examination

CODE_____ D.O.E._____

NAME _____ SEX___ RACE_____ D.O.B._____ AGE_____ G.A._____ BW _____

Posture and tone

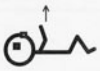
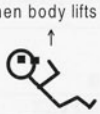
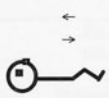
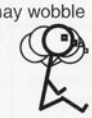
						STATE	ASYMM
POSTURE Infant supine. Look mainly at position of legs but also note arms. *Score predominant posture.*	arms & legs extended or very slightly flexed 	Legs slightly flexed 	legs well flexed but not adducted 	legs well flexed & adducted near abdomen 	abnormal posture: a) opisthotonus b) marked leg extension, strong arm flexion 		
ARM RECOIL Take both hands, quickly extend arms parallel to the body, Count to three. Release. Repeat 3 times.	arms do not flex 	arms flex slowly, not always; not completely 	arms flex slowly; more completely 	arms flex quickly and completely 	arms difficult to extend; snap back forcefully		
ARM TRACTION Hold wrist and pull arm upwards. Note flexion at elbow and resistance while shoulder lifts off table. *Test each side separately.*	arms remain straight; no resistance felt  R L	arms flex slightly or some resistance felt  R L	arms flex well till shoulder lifts, then straighten  R L	arms flex at approx 100° & maintained as shoulder lifts  R L	flexion of arms <100°; maintained when body lifts up  R L		
LEG RECOIL Take *both* ankles in one hand, flex hips + knees. Quickly extend. Release. Repeat 3 times.	No flexion 	incomplete or variable flexion 	complete but slow flexion 	complete fast flexion 	legs difficult to extend; snap back forcefully		
LEG TRACTION Grasp ankle and slowly pull leg upwards. Note flexion at knees and resistance as buttocks lift. *Test each side separately.*	legs straight - no resistance felt  R L	legs flex slightly or some resistance felt  R L	legs flex well till bottom lifts up  R L	knee flexes remains flexed when bottom up  R L	flexion stays when back+bottom up  R L		
POPLITEAL ANGLE Fix knee on abdomen, extend leg by gentle pressure with index finger behind the ankle. Note angle at knee. *Test each side separately.*	 180° R L	 ≈150° R L	 ≈110° R L	 ≈90° R L	 <90° R L		
HEAD CONTROL (1) ***(extensor tone)*** Infant sitting upright. Encircle chest with both hands holding shoulders. Let head drop forward.	no attempt to raise head 	infant tries: effort better felt than seen 	raises head but drops forward or back 	raises head: remains vertical; it may wobble 			
HEAD CONTROL (2) ***(flexor tone)*** Infant sitting upright. Encircle chest with both hands holding shoulders. Let head drop backward.	no attempt to raise head 	infant tries: effort better felt than seen 	raises head but drops forward or back 	raises head: remains vertical; it may wobble 	head upright or extended; cannot be passively flexed		
HEAD LAG Pull infant towards sitting posture by traction on both wrists & support head slightly. Also note arm flexion.	head drops & stays back 	tries to lift head but it drops back 	able to lift head slightly 	lifts head in line with body 	head in front of body		
VENTRAL SUSPENSION Hold infant in ventral suspension. Observe back, flexion of limbs, and relation of head to trunk. If it looks different, DRAW.	back curved, head & limbs hang straight	back curved, head ↓, limbs slightly flexed	back slightly curved, limbs flexed	back straight, head in line, limbs flexed	back straight, head above body		

Fig. 130.3 Hammersmith Neurological examination. Reproduced from [4] with permission (*cont.*)

Tone patterns

FLEXOR TONE (1) **(on traction: arm versus leg)** Compare scores of arm traction with leg traction.		score for arm flexion less than leg flexion	score for arm flexion equal to leg flexion	score for arm flexion more than leg flexion but difference 1 column or less	score for arm flexion more than leg flexion but difference more than 1 column		
FLEXOR TONE (2) **(arm versus leg)** Posture in supine.			arms and legs flexed	strong arm flexion with strong leg extension *intermittent*	strong arm flexion with strong leg extension *continuous*		
LEG EXTENSOR TONE Compare scores of leg traction and popliteal angle.		score for leg traction more than score for popliteal angle	score for leg traction equal to score for popliteal angle	score for leg traction less than score for popliteal angle, by 1 column only	score for leg traction less than score for popliteal angle, by more than 1 column		
NECK EXTENSOR TONE (SITTING) Compare scores of head control 1 and 2.		score for head extension less than head flexion	score for head extension equal to head flexion	score for head extension more than head flexion. but difference 1 column or less	score for head extension more than head flexion but difference more than 1 column		
INCREASED EXTENSOR TONE (HORIZONTAL) Compare scores of head lag and ventral suspension.		score for ventral suspension less than head lag	score for ventral suspension equal to head lag	score for ventral suspension more than head lag but difference 1 column or less	score for ventral suspension more than head lag but difference more than 1 column		

Reflexes

TENDON REFLEX Test biceps, knee, and ankle jerks.	absent	felt, not seen	seen	"exaggerated" (very brisk)	clonus		
SUCK / GAG Little finger into mouth with pulp of finger upwards.	no gag / no suck	weak irregular suck only No stripping	weak regular suck Some stripping	strong suck: (a) irregular (b) regular Good stripping	no suck but strong clenching		
PALMAR GRASP Put index finger into the hand and gently press palmar surface. Do not touch dorsal surface. *Test each side separately.*	no response R L	short, weak flexion of fingers R L	strong flexion of fingers R L	strong finger flexion, shoulder ↑ R L	very strong grasp; infant can be lifted off couch R L		
PLANTAR GRASP Press thumb on the sole below the toes. *Test each side separately.*	no response R L	partial plantar flexion of toes R L	toes curve around the examiner's finger R L				
PLACING Lift infant in an upright position and stroke the dorsum of the foot against a protruding edge of a flat surface. *Test each side separately.*	no response R L	dorsiflexion of ankle only R L	full placing response with flexion of hip and knee & placing sole on surface R L				
MORO REFLEX One hand supports infant's head in midline, the other the back. Raise infant to 45° and when infant is relaxed let head fall through 10°. Note if jerky. Repeat 3 times.	no response, or opening of hands only	full abduction at shoulder and extension of the arms; no adduction	full abduction, but only delayed or partial adduction	partial abduction at shoulder, and extension of arms followed by smooth adduction	• minimal abduction or adduction • no abduction or adduction; only forward extension of arms • marked adduction only or		

Fig. 130.3 (*cont.*)

the infant's attention span is relatively independent from other subsystems and the baby can use it to regulate and control investigation of the environment, i.e., by approximately one month post-term for a healthy, full-term infant [6, 16]. The assessment is used as a starting point to plan individualized and family focused intervention strategies. The Neonatal Individualized Developmental Care Assessment Program (NIDCAP) is at present the most comprehensive and advanced method to promote individualized developmental supportive and family-centered care in neonatal intensive care units [15, 16].

Movements

SPONTANEOUS MOVEMENT (quantity) Watch infant lying supine.	no movement	sporadic and short isolated movements	frequent isolated movements	frequent generalized movements	continuous exaggerated movements		
SPONTANEOUS MOVEMENT (quality) Watch infant lying supine.	only stretches	stretches and random abrupt movements; some smooth movements	fluent movements but monotonous	fluent alternating movements of arms + legs; good variability	• cramped, synchronized; • mouthing • jerky or other abnormal movements		
HEAD RAISING PRONE Infant in prone, head in midline.	no response	infant rolls head over, chin not raised	infant raises chin, rolls head over	infant brings head and chin up	infant brings head up and keeps it up		

Abnormal signs/patterns

ABNORMAL HAND OR TOE POSTURES		hands open, toes straight most of the time	intermittent fisting or thumb adduction	continuous fisting or thumb adduction; index finger flexion, thumb opposition	continuous big toe extension or flexion of all toes		
TREMOR		no tremor, or tremor only when crying or only after Moro reflex	tremor occasionally when awake	frequent tremors when awake	continuous tremors		
STARTLE	no startle even to sudden noise	no spontaneous startle but reacts to sudden noise	2-3 spontaneous startles	more than 3 spontaneous startles	continuous startles		

Orientation and behaviour

EYE APPEARANCES	does not open eyes		full conjugated eye movements	*transient* • nystagmus • strabismus • roving eye movements • sunset sign	*persistent* • nystagmus • strabismus • roving eye movements abnormal pupils		
AUDITORY ORIENTATION Infant awake. Wrap infant. Hold rattle 10 to 15 cm from ear.	no reaction	auditory startle; brightens and stills; no true orientation	shifting of eyes, head might turn towards source	prolonged head turn to stimulus; search with eyes; smooth	turns head (jerkily, abruptly) & eyes towards noise every time		
VISUAL ORIENTATION Wrap infant, wake up with rattle if needed or rock gently. Note if baby can see and follow red ball (B) or target (T).	does not follow or focus on stimuli B T	stills, focuses, follows briefly to the side but loses stimuli B T	follows horizontally and vertically; no head turn B T	follows horizontally and vertically; turns head B T	follows in a circle B T		
ALERTNESS *Tested as response to visual stimuli* (B or T).	will not respond to stimuli	when awake, looks only briefly	when awake, looks at stimuli but loses them	keeps interest in stimuli	does not tire (hyper-reactive)		
IRRITABILITY In response to stimuli.	quiet all the time, not irritable to any stimuli	awakes, cries sometimes when handled	cries often when handled	cries always when handled	cries even when not handled		
CONSOLABILITY Ease to quiet infant.	not crying; consoling not needed	cries briefly; consoling not needed	cries; becomes quiet when talked to	cries; needs picking up to be consoled	cries; cannot be consoled		
CRY	no cry at all	whimpering cry only	cries to stimuli but normal pitch		High-pitched cry; often continuous		

SUMMARY OF EXAMINATION:

HEAD AND TRUNK TONE: LIMB TONE:

MOTILITY: REFLEXES:

ORIENTATION AND ALERTNESS: IRRITABILITY:

CONSOLABILITY: LIST DEVIANT SIGNS:

Fig. 130.3 (*cont.*)

Dubowitz and Dubowitz [17], and Dubowitz et al [4, 18] developed an examination that included the assessment of neurological and behavioral items, taking into account behavioral states [4, 17, 18]. The authors stated that "ideally, a meaningful neurological assessment should be part of the routine clinical examination of every newborn infant". One of the strengths of this examination is that it is suitable for preterm infants as well for full-term infants, and for both ill and healthy infants. It is a simple, objective recording system, based on drawings and diagrams, recorded on a pro-forma that includes definitions. The diagrams are easy to tick and are suitable for staff with no particular expertise or experience in neonatal neurology. The examination does not take more than 15 minutes to perform and can be used as part of the routine clinical assessment of newborn infant. It is also suitable for repeated examinations. It is therefore possible to document the normal or abnormal evolution of neurological behavior in the preterm infant after birth, to compare this behavior with that expected in newborn infants of corresponding postmenstrual age and to detect features of a neonatal encephalopathy. The examination includes assessment of behavioral states, tone, and primitive reflexes and also motility and some aspects of behavior. One advantage of this method has been the development of an optimality score that allow quantification of the deviant scores and use in different settings. Another advantage is that authors have updated the examination and have provided some general guidelines on the most common findings at each gestational age (Fig. 130.3). The examination has been validated in full-term and preterm infants [4, 18].

130.3 Spontaneous Movement Patterns and General Movement

The general movements (GMs) method was the last scheme of assessment of the nervous system to be standardized and validated. It was conceived by Prechtl and co-workers during the late eighties [11, 19–21]. It is based on the observation of spontaneous movements of the fetus and of the newborn infant, either preterm or full-term. Since the seventies, Prechtl and co-worker had focused their attention on the spontaneous movements of the fetus observed using ultrasound scans: they recognized that spontaneous movements could be distinguished as movements clearly constant in form and therefore easily recognisable. Prechtl defined these sequences as "movement patterns". With the aid of ultrasound he was able to recognize several fetal movements patterns such as startles, GMs, isolated limb movements, twitches, stretches, breathing movements, hiccups, yawns, head rotations, head flexion, sucking, swallowing and others [20]. They emerge as early as 9–12 weeks postmenstrual age (Table 130.1). It is striking that they look complex and differentiated from their first appearance. They hardly show any change in form in the first weeks after birth, despite the profound changes in the environmental conditions; thus, an intrauterine or extrauterine environment seems to influence them very little. Two of these movement patterns (stretches and yawns) are maintained throughout life without changing form. Surprisingly, local and isolated movements of the limbs appear 1–2 weeks sooner than GMs. They continue to be present during the whole preterm period and are seen until the age of 5–6 months post-term. The immature nervous system of the fetus generates these movement patterns without being stimulated: they are generated endogenously, and reflect spontaneous activity of the brain.

The chance to observe, record and eventually measure endogenously generated brain activity is one of the dreams of neurobiologists. Prechtl's idea was that spontaneous movements were markers of brain impairment and dysfunction [14].

It is likely that GMs are produced by complex nervous networks, the so-called central pattern generators (CPG) that are located in different parts of the brain and at different brain levels, especially in the higher parts of medulla and the brain stem. Breathing, sucking, chewing, eye movements, swimming, crawling and walking are other spontaneous motor activities that appear to be endogenously generated, i.e., generated

Table 130.1 Fetal motor repertoire age is given in postmenstrual age. Reproduced from [20], with permission.

10 weeks	12 weeks	14 weeks	20 weeks
Startles	Startles	Startles	Startles
GMs	GMs	GMs	GMs
Isolated arm movements	Isolated arm movements	Isolated arm movements	Isolated arm movements
Isolated leg movements	Isolated leg movements	Isolated leg movements	Isolated leg movements
Hiccup	Hiccup	Hiccup	Hiccup
	Breathing movements	Breathing movements	Breathing movements
	Hand-face contact	Hand-face contact	Hand-face contact
	Head retro-flexion	Head retro-flexion	Head retro-flexion
	Head ante-flexion	Head ante-flexion	Head ante-flexion
	Head rotation	Head rotation	Head rotation
	Stretch	Stretch	Stretch
	Yawn	Yawn	Yawn
		Sucking and swallowing	Sucking and swallowing
			Eye movements

without any recognizable external stimulus. The combination of these motor activities varies depending on the ongoing behavioral state. During state 2 (irregular breathing), slow and rapid eyes movements and body movements are fired by CPG (termed "active" or "agitated" sleep). During state 1 (quiet sleep), there is regular breathing with no eye and no body movements, reflecting the different neural mechanisms that actively inhibit (or modulate in the case of respiration) these motor activities from higher cortical and sub-cortical structures. GMs as well as the other movement patters are typical of states 2, 4 and 5. Startling which appears in state 1, is an exception [19, 21].

130.4 What Are General Movements?

General movements (GMs) consist of movements that involve the whole body in a variable sequence of arm, leg, neck and trunk movements. They wax and wane in intensity, force and speed, with a gradual beginning and end. Rotation along the axis of the limbs and continuous changes in the direction of movement make them fluent and elegant and create an impression of complexity and variability. Minor changes can be seen during early develppoment: preterm, GMs are of large amplitude and often accompanied by lifting of the pelvis; at term, GMs are smaller in amplitude and show a writhing character that gradually disappears; at 6–9 weeks post-term, fidgety GMs start to appear [11, 22].

GMs are the most frequent of the various movement patterns exhibited by newborn babies, and also the most complex. It is likely that their complexity makes them vulnerable to brain dysfunction, and therefore a sensitive indicator of brain injury.

Brain lesions affect the quality rather than the quantity of GMs. The only exception to this rule is that severe perinatal asphyxia is accompanied by a transient phase of hypokinesia. Other brain lesions are associated with preterm and writhing GMs changing in character with loss of fluency, variability and complexity, with a poor repertoire and becoming cramped-synchronized or chaotic. Abnormal fidgety GMs may be either exaggerated or absent [22, 23].

Poor repertoire GMs are the most common abnormality and variability in sequence is lost or poor. When abnormal GMs are followed by normal fidgety movements, recovery from brain lesions and a normal outcome are expected. However, absence of fidgety movements is likely to herald cerebral palsy [24]. Cramped synchronized GMs also represent a severe GM abnormality and are recognized when all limbs and trunks muscle contract and relax almost simultaneously (Fig. 130.4) [23]. If this abnormal character of GMs persists for weeks and is accompanied and/or followed by no fidgety movements, the development of a spastic form of cerebral palsy is predictable (Fig. 130.5) [22–24].

130.5 The Neurological Examination in the Sick Newborn Infant

Sick infants should be have a daily neurological examination. Tthis should be with minimal handling if there is cadiorespiratory instability. The neurological examination is hampered by long lines, monitoring and sedation, in which

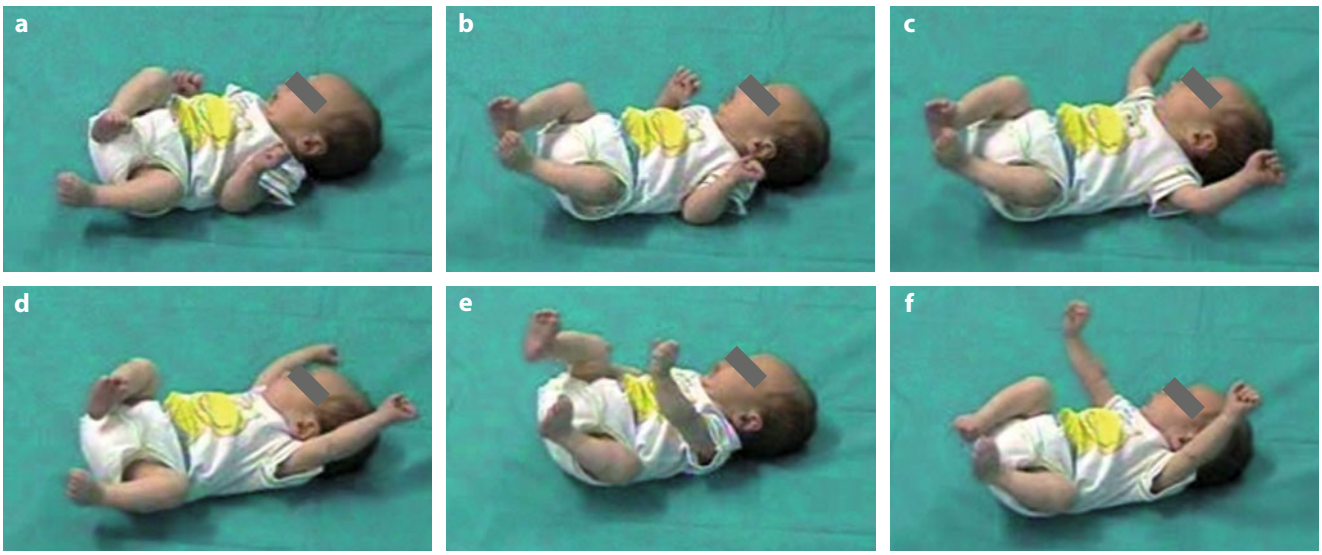


Fig. 130.4 A cramped synchronized GM: the six pictures show synchronous lifting of the four limbs in sequence, followed by extension and adduction

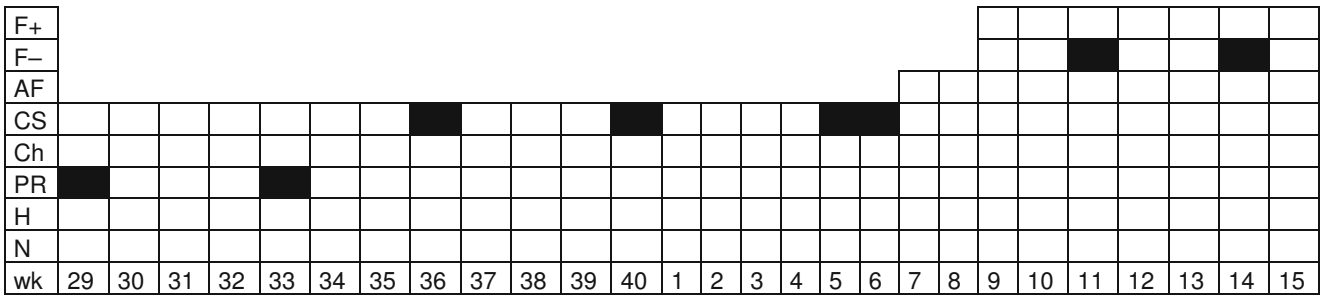


Fig. 130.5 Individual developmental trajectory. Trajectory of a preterm infant, born at 27 weeks postmenstrual age. PR GMs in the preterm age, CS at term and early post-term age are followed by absent fidgety movement and subsequent cerebral palsy.Poor repertoire (PR), cramped synchronised (CS), hypokinesia (H), chaotic movements (Ch), abnormal fidgety movements (AF), fidgety movements (F+), absent fidgety (F-)

Name ..... Date of birth ..... Gestational age .....
Number of the assessment ..... Date ..... Hours/days of life .....
Examiner .....

N	ITEMS	SCORE 0	SCORE 1	SCORE 2
1	**Respiration**	Normal	Ataxic/periodic Polipnea Apneas	Absent
2	**Posture**	Variable	No variable posture Exaggerated flexion/extension	Decerebrated/decorticated Stiff in flexion Flat on the mattress
3	**General Movements**	Present	Hypokinesis/poor repertoire GMs	Absent/cramped synchronized/chaotic GMs
4	**Eye movements**	Present	Rare	Absent/eye deviation
5	**Resistance to passive movs:** **Neck** **Trunk** **Limbs**	 Normal Normal Normal	 Poor/exaggerated Poor/exaggerated Poor/exaggerated	 Severely reduced/increased Severely reduced/increased Severely reduced/increased
6	**Motor response to pain**	Present	Reduced	Absent
7	**Pupils**	Normal	Mydriasis or myosis, reactive to light	No response to light
8	**Brainstem reflexes:** **Sucking** **Corneal** **Oculocephalic**	 Present Present Present	 Poor Poor Poor	 Absent Absent Absent
9	**Seizures** (clonic jerks, tonic, subtle) **or motor automatisms** (rowing, pedalling)	Absent	Isolated	Frequent

Annotations:.....
.....
.....
....

Fig. 130.6 Neurological assessment of the sick newborn infant

case the examination may be curtailed to a few items that can be assessed reasonably quickly. The baby's level of consciousness should be assessed. The general use of cerebral ultrasound and MRI scans enable documentation of the presence, evolution and severity of brain lesions.

New neuroprotective strategies such as brain cooling require the recognition of a severe neonatal encephalopathy (NE) soon after birth. A neurological scheme for assessing the severity and evolution of acute brain dysfunction is described in Fig. 130.6. It is based on nine items, mostly requiring observation of spontaneous movements. It includes assessing the level of consciousness (motor response to pinch of the skin of the leg, pupillary size and reaction to light), respiration, posture, spontaneous general movements and eye movements, brainstem reflexes (corneal and oculocephalic), resistance of the trunk, neck and limbs to passive movement, and abnormal movements (seizures and or limb automatisms, such brain stem release phenomena, BSRP).

130.6 Hyypoxic Ischemic Events and NE in the Full-Term Infant

NE is a clinical syndrome of disturbed neurological function. It is manifested by difficulty in the initiation and maintenance of respiration, depression of tone and reflexes, abnormal level of consciousness, and often seizures [25]. NE may be caused by perinatal asphyxia, as well as other conditions such as stroke, infection, mitochondrial disease, brain malformation, and metabolic disorders.

Volpe has provided an accurate description of the development of NE due to perinatal asphyxia (HIE) in full-term infants [1]. From birth to 12 hours, severely affected infants are stuporose or comatose (i.e., not arousable and with minimal or no response to sensation), have periodic breathing or respiratory irregularity, intact pupillary responses and spontaneous eye movements. Infants are hypotonic, and have minimal spontaneous or elicited movements and hypokinesia is common. Very few babies show signs of brain stem injury, such as pupils that are fixed and in the midposition and dilated, and eye movements, which are fixed when the doll's eye maneuver is performed.

50–60% of NE infants who ultimately convulse have seizures, which are mostly subtle, by 6–12 hours after birth. By 12–24 hours, the infant's level of consciousness may appear to improve and there are severe seizures with overt status epilepticus, apneic spells, jitteriness and weakness in a hip-shoulder distribution. Babies with basal ganglia (BG) injury may have spontaneous or evoked hypertonus. Between 24 and 72 hours, the baby with NE may be in deep stupor or coma, with irregular ataxic respirations (and even respiratory arrest), brain stem oculomotor and papillary disturbances disturbances (i.e., constricted hyporeactive pupils, fixed to light dilated pupils) [1].

Sarnat and Sarnat in 1976 [5] developed a clinical grading system for the severity and the progression of the neurological signs in HIE. Stage 1 lasted less than 24 hours, characterized by hyperalertness, uninhibited Moro and stretch reflexes, sympathetic effects, and a normal electroencephalogram. Stage 2 is chacterised by obtundation, hypotonia, strong distal flexion, and multifocal seizures. The electroencephalogram (EEG) shows a periodic pattern, sometimes preceded by continuous delta activity. Infants in stage 3 are stuporous, flaccid, with suppressed brain stem and autonomic functions. The EEG is isoelectric or with infrequent periodic discharges. Amiel-Tison [26], Finer [27], Fenichel [28], Levene [25] and others developed alternative scoring systems along similar lines. All accepted the Sarnat three grade system, from 1 (mild) to 3 (severe). In spite of some differences, they agree on the prognostic value of HIE: grade 1 correlates with a good outcome, grade 3 with poor outcome, and grade 2 with neurological sequelae affecting 25–40% of cases.

Recent MRI studies have demonstrated that in acute severe global hypoxic ischemia, the basal ganglia (BG) and thalami (T) are mainly affected. Lesions in these areas are often associated with abnormal signal intensity in the posterior limb of the internal capsule (PLIC). Concomitant injury to the cortex (C) of the central sulcus and the adjacent sub-cortical white matter (WM) is common. In severe cases, the brainstem may also be involved. When the predominant lesion involves WM and cortex but spares the BGT and PLIC, the likely cause is prolonged partial hypoxia-ischemia [1, 19, 23]. The clinical correlates of these lesions during the first hours of life are not known. Future studies based on early diffusion weighted imaging and early serial neurological examinations may provide an answer. Some signs are markers of specific brain structure involvement. Seizures with clonic focal or multifocal jerks are likely to rise from damage to the cortex and white matter. Akinesia (no eye movement), divergent eye position, unresponsive pupils, monotonous respiration and brainstem release phenomena, are markers of brain stem involv- ement and/or BGT and PLIC lesions. Coma or severe stupor, accompanied by tonic seizures, BSRP and lack of body and eye movements indicate a more severe and diffuse brain involvement as in the most serious MRI pattern seen after perinatal asphyxia with lesions in the BGT, PLIC, WM and C [1, 23].

130.7 Other Brain Injuries

130.7.1 Periventricular Leukomalacia (PVL)

The initial neurological correlates of PVL are not establish: cystic or noncystic PVL takes 2–4 weeks to become manifest after US has demonstrated increased periventricular echogenicity, which is clinically silent. Other hemorrhagic, metabolic or infective disorders may be associated. Several clinical signs have been described: in preterm infants, who

later develop cerebral palsy, signs such as irritability, abnormal posture of fingers, spontaneous Babinski reflex, popliteal angle reduction, increased extensor tone, inferior limbs weakness and marked hypotonia have been observed. Long-term correlates of PVL include spastic diplegia and intellectual deficits [1, 18].

130.7.2 Intraventricular Hemorrhage (IVH) in Preterm Infants

Three clinical syndromes are associated with IVH:

- Catastrophic clinical deterioration, which is a dramatic presentation usually in the most severe degrees of IVH. It is characterized by deep stupor and coma, respiratory abnormalities (hypoventilation and apneas), seizures, decerebrate posturing, pupils fixed to light, and eyes fixed to vestibular stimulation, and by a flaccid quadriparesis
- Saltatory syndrome, which is most subtle in presentation and evolves over many hours. Signs are: altered consciousness, decreased quantity and quality of spontaneous movement, altered of ocular position and motility.
- A clinically silent syndrome, when neurological signs are so subtle that they are not seen [1, 15].

130.7.3 Bilirubin Encephalopathy

The clinical features in infants with bilirubin injury depend on the damage topography and on the infant age. Acute bilirubin encephalopathy is characterised by three phases:

- In the first phase, stupor and hypotonia are prominent, often accompanied by poor sucking. Seizures occur in a minority of cases.
- In the second phase, the principal signs are hypertonia, particularly in the extensor muscles with backward arching of the neck (retrocollis) or of the back (opisthotonus). The increase in tone is probably of extrapyramidal origin and newborns, who show these signs, later develop features of chronic bilirubin encephalopathy.
- In the third phase, hypertonia evolves into hypotonia, usually after the first week of life [1].

130.7.4 Metabolic Disease

Metabolic disorders during the neonatal period are generally associated with: altered level of consciousness, seizures, hypotonia or hypertonia, impaired feeding, and delayed neurological development. Stupor, coma and seizures, urine with the odour of maple syrup occurs in maple syrup disease. Seizures, stupor or coma associated with myoclonus and hiccups are typical of nonketotic hyperglycinemia. Hyperammonemic states are accompanied by vomiting, poor feeding, stupor, coma. There are dysmorphic facial features in some metabolic diseases such as the mitocondriopathies, which may manifest in the neonatal period with seizures, hypotonia, stupor, coma and tachypnea due to a metabolic acidosis [1].

130.7.5 Jitteriness

Jitteriness is a disorder of movement frequently observed during the neonatal period. Movements are generalized and symmetrical, have the quality of a coarse tremor, are stimulus sensitive and can be diminished by gentle flexion of the limbs. It is frequently accompanied by brisk tendon reflex and an easily elicited Moro reflex (hyperexitability syndrome). Jitteriness is often related to neurologic al hyperirritability, as in f HIE, hypoglycemia, hypocalcemia, drug withdrawal syndrome,. In some cases, no cause can be found [1, 13].

130.7.6 Stiff Baby Syndrome

Stiff baby syndrome or hyperexplexia is a familiar disorder (autosomic dominant). It is characterised by marked hypertonia, which is accentuated by minor sensory stimulation. An episode of hypertonia may involve respiratory muscles, causing apnea. Hypertonia is accompanied by an exaggerated startle response, which may mimic seizures [1].

130.7.7 Neuromuscolar Disorders or Spinal Cord Disease: the Floppy Infant

The term floppy baby or infant is used to denote an infant with poor muscle tone affecting the limbs, trunk and the cranio-facial musculature. Hypotonia may be caused by congenital or acquired disorders, as a consequence of central or peripheral nervous system involvement. Lower motor neuron lesions result in a loss of muscle tone and flaccidity. Lesions affecting the upper motor neurons result in reduced supraspinal inhibitory influences, leading during early infancy to flaccidity and ldiminished muscle tone with preserved or hyperactive reflexes, and later evolving into spasticity. Thus hypotonia with no obvious weakness and normal or increased reflexes suggests CNS involvement. In neuromuscolar disorders, hypotonia is usually associated with weakness and/or contractures. Contractures, skin dimpling and poor dermatoglyphic patterns are indicators of poor fetal movements and are highly suggestive of a neuromuscular disorder. In myasthenia gravis and infantile botulism, diffuse hypotonia and weakness are present, often in conjunction with CNS involvement. In congenital myopathies, proximal extremity weakness is prominent and there are limb deformities if the onset was *in utero*.

Spinal cord trauma with a tear in the cervical dura can be caused by a difficult breech delivery. This results in symmetric lower extremity paralysis with sparing of the face and cranial nerves and involvement of the sphincters. The most common injury involving the peripheral nerves is the proximal cervical roots of C5, C6, and C7, usually caused by a traumatic delivery with shoulder dystocia. In Erb's palsy, there is paralysis of shoulder abduction, elbow flexion, and finger extension, so that the arm is held extended, externally rotated with flexion at the wrist. There is no biceps reflex,although the triceps reflex may be present [1].

130.8 Conclusions

Advances in neuroimaging and other diagnostic techniques do not reduce the importance of performing a neurological examination, which may highlight the presence of neurological abnormalities and help to select those infants who need investigation, neuroprotection and follow-up.

A full neurological examination will show if the development of the baby is appropriate for postmenstrual age.

Sick newborn infants require a simple non-invasive test to evaluate the state of consciousness, and the severity of the brain damage. If there is coma or stupor, brainstem involvement should be considered and the neurological test should be repeated every few hours.

Seizures are the most important sign of an acute brain disorder. They may be difficult to recognize without concomitant EEG recording. Only clonic jerks are easily recognizable, indicating acute cortical involvement. Spontaneous movements and GMs in particular are reliable and effective tools for the diagnosis of brain dysfunction and may predict the motor outcome.

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131

Neonatal Electroencephalography

Lena K. Hellström-Westas

131.1 The Neonatal Electroencephalogram

The electroencephalogram (EEG) reflects brain function and is an important diagnostic tool for infants requiring neonatal intensive care, not least since neurological symptoms may be very vague, or entirely absent. The EEG can be used for early detection of general or focal cerebral abnormalities, and also to give important predictive information on later neurodevelopmental outcome.

The standard EEG is recorded from electrodes placed over the scalp according to the International 10–20 system which uses the nasion and the inion as anatomical landmarks on the skull to identify electrode positions. The inter-electrode distance is usually 10 or 20% of the total distance between these positions. The electrode positions are defined by capital letters followed by a number, e.g., F for frontal, C for central, T for temporal, O for occipital, with even numbers indicating the right side and odd numbers the left side. Additionally, respiration, electrocardiogram, eye movement and electromyogram are recorded, which together with the EEG give supplementary information such as sleep states, movements, seizures, and possible artefacts. EEG development occurs mainly in parallel in the fetus and in the preterm infant with comparable maturation. The terms postconceptional age (PCA), or postmenstrual age (PMA), represent a summary of gestational age and postnatal age and are used when assessing EEG maturation.

131.1.1 Normal EEG Maturation

The recorded EEG can be described according to amplitude, frequency, topographic distribution of activity and temporal (time) evolution. The normal EEG background in very preterm infants is mainly discontinuous, a pattern that is called *tracé discontinu* which is characterized by high-amplitude bursts of activity alternating with periods of low-voltage activity, interburst intervals (IBI). With increasing maturation the duration of the bursts increase while their amplitude decreases, and simultaneously the IBIs become shorter, resulting in an increasingly continuous EEG background. The mean IBI duration is around 18–26 seconds at 21–24 weeks PCA and decreases to 10–12 seconds at 25–30 weeks PCA, and down to 6–8 seconds at 34–36 weeks. The topographic organization of the developing EEG occurs in an occipital-frontal direction. Interhemispheric synchrony of electrocortical activity changes during maturation: at extremely low PCA the EEG activity is usually synchronous, between 26 and 30 weeks PCA there is increased desynchronization, which again is followed by increasing synchronization at PCAs above 30 weeks [1–3].

Sleep wake cycling can be identified in the EEG from around 30 weeks' PCA, but already from around 24–25 weeks PCA immature cycling can be seen although specific sleep states cannot be distinguished. Active sleep, or rapid eye movement (REM) sleep, can usually not be distinguished from wakefulness in the EEG without other measures, i.e., direct observation or evaluation of eye movements, respiration and muscular activity. Quiet sleep, or non-REM, sleep is characterized by increased discontinuity. The EEG during quiet sleep in term infants is either discontinuous (*tracé alternant*), or contains high voltage slow activity (HVS).

The distribution of the main EEG frequencies, i.e., delta (< 4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (13–20 Hz), also change with maturation, although there is usually a mix of frequencies at all PCA. In preterm infants, high voltage delta activity, and to some extent, theta activity is predominant while theta and alpha activity can be identified in term infants [1–3]. Recently, the presence of very slow EEG activity (< 0.5 Hz) has been demonstrated both in term and preterm infants. Experimental data indicate that this activity may be essential for development of brain wiring processes [4]. High frequency oscillation ventilation (HFOV) in the

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NICU is often performed with frequencies between 10 and 15 Hz, i.e., frequencies that are parts of the normal EEG, and care has to be taken to avoid such interference. When EEG-trend monitoring (see below) is performed during HFOV, it is important to avoid contact between the electrodes and the bedding, and to evaluate the raw-EEG signal.

During normal EEG maturation, several distinctive waveforms and patterns are expected to appear and disappear at certain PCA: these can be used for evaluation of maturation although their functional and anatomical correlates are not known. Delta brush pattern is characterized by runs of fast activity (alpha-beta) superimposed on delta waves; this pattern is typical of the very preterm EEG. Temporal theta bursts (temporal saw-tooth) are brief, rhythmic, 4–6 Hz transients that first appear around 26 weeks PCA and reach a maximum at 30–32 weeks before they disappear. Temporal sharp waves may appear during the first weeks of life in infants born at 31–32 weeks gestation. However, if abundant, or persisting, they are associated with brain injury. Frontal sharp wave transients appear from 34–35 weeks PCA and disappear around 10 weeks later [1–3].

The accuracy of estimation of PCA from various EEG measures is usually 2 weeks. An interesting observation in a few publications is that the EEG activity in very preterm infants seems to exhibit a progressive increase during the first days of life: whether this is due to postnatal adaptation or recovery is not known [5].

131.1.2 Abnormal EEG

Severe hypoxic-ischemic or metabolic insults such as perinatal asphyxia, hypoglycemia or severe hypotension are associated with transient EEG depression. The electrocortical activity usually recovers after an insult during a time-period that may last from minutes to weeks. Seizures of varying intensity often develop during the recovery phase [6]. Sleep-wake cycling is usually depressed or entirely absent during the acute stage. Chronic EEG changes may persist as markers of permanent damage. The consistent way by which the electrocortical activity behaves after an insult, and the relation between the severity of the EEG activity and the brain damage, makes EEG sensitive for diagnosis of brain damage and early prediction of outcome.

Acute changes during and after an insult are characterized by slowing of the EEG, amplitude depression and increasing discontinuity of varying degree and duration, which correlates with the severity of the insult. A severe insult may result in an entirely flat (inactive, isoelectric) EEG, while mild insults may only results in subtle slowing of the EEG or increased discontinuity. Burst-suppression (BS) and undifferentiated extremely low voltage patterns may also be present after severe insults. These EEG patterns are both associated with increased risk for adverse outcome in asphyxiated term infants.

In the very preterm infant, the IBIs may become prolonged, and the background may also change to a BS pattern. Burst-suppression is characterized by bursts of activity with flat (inactive) or very low amplitude interburst intervals, as compared to the normal discontinuous background of the preterm infant in which the IBIs contain more activity. The BS pattern is abnormal at all maturational stages; it is associated with presence of brain damage, administration of sedative medications, and some rare encephalopathies (mainly non-ketotic hyperglycinemia and Ohtahara syndrome). In term infants, a recording with predominant IBI duration of more than 30 seconds, is highly predictive of death or poor neurological outcome [7].

Chronic stage EEG changes appear within a few weeks after an insult and are markers of persisting brain injury. Chronic stage background abnormalities can be categorized as being either dysmature or disorganized. Dysmature EEG patterns are characterized by delayed development (> 2 weeks) and are associated with cognitive impairment. Disorganized EEG patterns develop after severe acute insults, and are characterized by deformity of delta waves and brushes, as well as presence of abnormal sharp waves; they are closely associated with white matter damage and development of cerebral palsy [8]. Some waveforms, especially positive rolandic sharp waves (PSRW) are markers of white matter damage. PRSW are predictive of later cerebral palsy when appearing with a frequency of more than two per minute in the neonatal EEG [9].

131.1.3 Effects of Medications

Administration of several antiepileptic or sedative medications, including benzodiazepines (diazepam, midazolam), phenobarbital, opioids (morphine, fentanyl), and lidocaine is associated with transient depression of the EEG background for minutes to hours. Surfactant treatment has also been associated with a 10 minute profound aEEG depression immediately after administration in some infants, the reason for which is not known. The most common response to a loading dose of sedative or anticonvulsant medication in a term infant with normal continuous EEG background is a change to a moderately discontinuous EEG pattern.

Preterm infants and infants with severely compromised brain function tend to respond with more profound discontinuity, including burst-suppression. The EEG background activity will usually recover within 2–2.5 hours after a single dose of sedative medications in term infants [10, 11]. Repeated doses and continuous infusion of such medications may, of course, be associated with prolonged and more marked discontinuity.

131.1.4 Seizures

The electrographic appearance of neonatal seizures can be very variable as regards waveform morphology, frequency, localization and temporal evolution. A seizure pattern in the EEG is often defined as "a sudden, repetitive, evolving and stereotyped ictal pattern with a clear beginning middle and ending and a minimum duration of 10 seconds" [12].

Status epilepticus is usually defined as repetitive seizures with a duration of more than 30 minutes. Term infants tend to have a focal onset of seizures, while seizures in a preterm more often are regional [13]. Clinical seizure identification can be difficult, since ill newborn infants may have seizure-suspected movements without corresponding EEG seizure activity, but also because a majority of neonatal seizures are entirely subclinical.

In babies with electroclinical seizures, administration of antiepileptic drugs is associated with a phenomenon called electroclinical dissociation, i.e., the electroclinical seizures disappear while subclinical seizures persist.

131.2 EEG-monitoring

Video-EEG monitoring is the gold standard of EEG-monitoring. However, it is not available in all neonatal care units (NICUs), and it is often too complicated for the neonatal staff to interpret bedside on a 24 hour/7 day basis unless continuous service is provided from an EEG department. This has, so far, limited the use of video-EEG to complicated cases and research studies. In contrast, continuous EEG-monitoring with limited channels, especially the amplitude integrated EEG (aEEG), is increasingly used in many NICUs since the method is easy to apply and to interpret by the neonatal staff. Furthermore, the usefulness of the aEEG has been demonstrated in several studies in both term and preterm infants requiring intensive care. The aEEG is derived from the raw EEG; first the EEG signal is passed through an asymmetric filter which strongly attenuates low (< 2Hz) and high (> 15 Hz) EEG frequencies, then the signal undergoes rectification, semi-logarithmic display (to give better resolution within very low voltage activity), and time-compression. The resulting aEEG trend gives a good overview of changes in minimum and maximum electrocortical activity over hours and days [14] (Fig. 131.1). Maturational aEEG changes parallels the EEG development, which is expected since it is derived from the EEG. A main developmental feature in the aEEG trend is the progressive rise of the lower border (during the more discontinuous quiet sleep) with increasing gestational age. The first aEEG monitor was created in the 1960s by Prior and Maynard, and was called the Cerebral Function Monitor (CFM).

Several studies have shown that aEEG recorded within the first 3–6 hours after birth asphyxia is highly predictive of neurodevelopmental outcome [15]. Abnormal background patterns (BS, extremely low voltage, flat) are predictive of adverse outcome, while normal continuous or slightly discontinuous patterns are associated with normal outcome. Sleep wake cycling appearing before 36 postnatal hours is associated with better outcome in infants with moderate hypoxic-ischemic encephalopathy (HIE). These studies were all performed in asphyxiated non-cooled infants who did not receive treatment with hypothermia. It was recently shown that intervention with moderate hypothermia is associated with altered predictive value of the aEEG and EEG [16, 17]. A normal very early aEEG or EEG is still associated with good outcome, while delayed recovery of the aEEG or EEG background until 36–48 hours, and later appearance of sleep wake cycling may be associated with healthy recovery. Recent data also indicate that seizures may still be abundant in cooled asphyxiated infants [18].

The aEEG in preterm infants is also predictive of outcome. Several aEEG and EEG studies have shown correlations between degree of early background depression and severity of intraventricular hemorrhages (IVH). It was also shown that seizures, mainly subclinical, are not uncommon in these infants. A few studies indicate that the averaged burst counts, or IBI, during the first days of life is predictive of outcome in preterm infants with IVH [19]. However, also other factors seem to affect the aEEG/EEG background in preterm infants, e.g., cardiac output and blood pressure, and arterial carbon dioxide levels. Presence of sleep-wake cycling during the first week of life is usually indicative of good recovery.

Continuous EEG-monitoring can be very useful for early detection of seizures in high-risk infants. However, a reduced number of EEG electrodes will record fewer seizures than a full EEG. Nevertheless, it has been demonstrated that a single-channel EEG from bilateral central leads, or a two-channel aEEG/EEG will record around 75–80% of all seizures that can be detected by a full EEG [12, 13]. A reason for this is that at least 60–70% of neonatal seizures will appear in the central, temporal and parietal areas [13].

Seizure identification in the aEEG trend requires the electrographic features of the seizure to be clearly distinguishable from the overall background activity since this will produce a transient change (often a rise, rarely a brief decrease) in the aEEG pattern. Repeated seizures and status epilepticus will result in a pattern similar to a saw-tooth, while ongoing epileptic seizure activity may be difficult to distinguish since there is no major change in the aEEG trend (Fig. 131.2). Brief seizures (< 30 seconds) may be very difficult to identify in the compressed aEEG trend, and for this reason it is also important to review the raw-EEG for seizure activity. Some of the newer aEEG/EEG monitors have seizure alerts which can be useful for identification of brief seizures, although no seizure alarms have a 100% sensitivity.

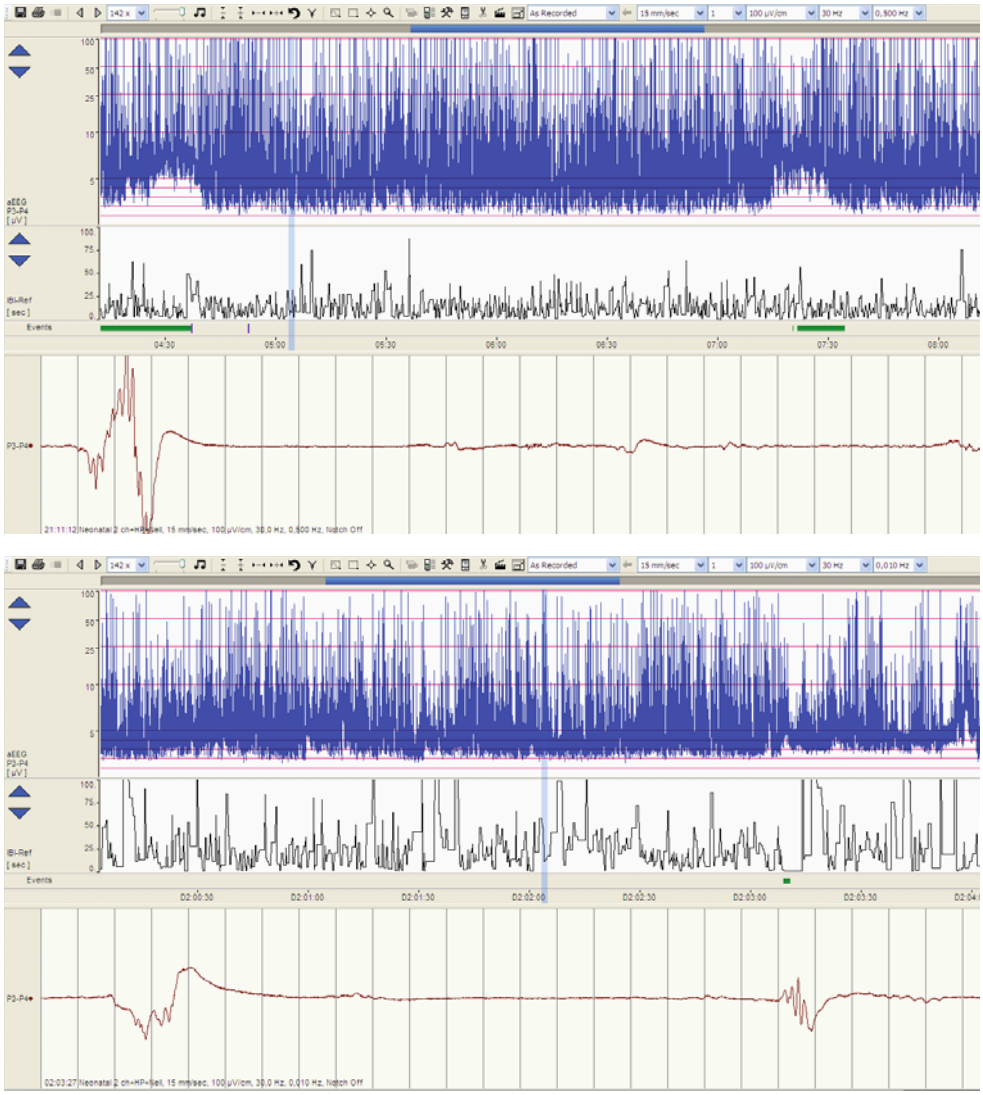

Fig. 131.1 Twins born at 24 gestational weeks, recorded during the first 2–3 days of life. Four hours of aEEG and IBI trends, below 25 seconds of corresponding EEG. The twin in the *upper panel* had a small unilateral IVH grade 1 and survived with normal outcome at 30 months (Bayley-II MDI and PDI scores >100). The twin in the *lower panel* developed bilateral IVH grade 3 and died during the first week of life. Note the differences in burst density, which are difficult to assess in the single channel EEG but visible in the aEEG trend, and better appreciated in the IBI trend. There are no seizures and no cyclicity present. The transient increases in the aEEG trend in twin a are due to care procedures

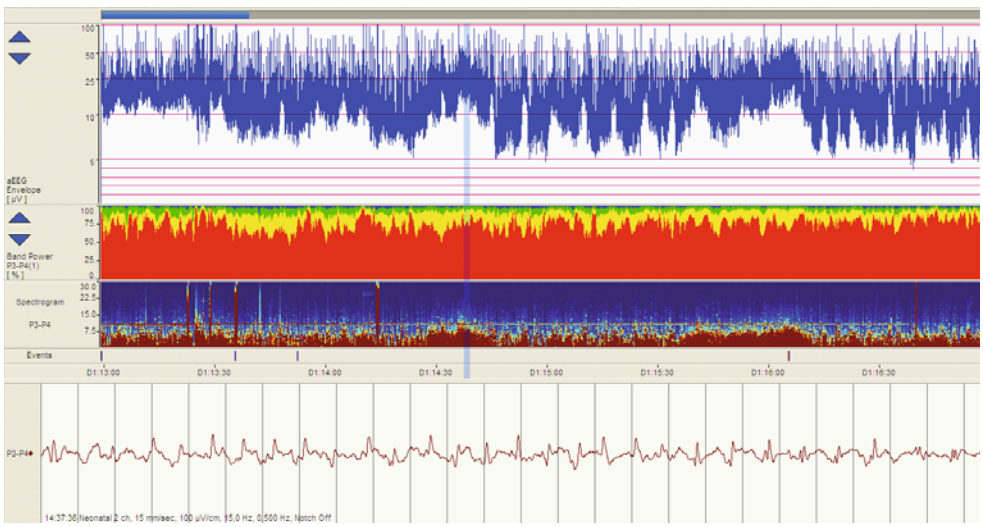

Fig. 131.2 Subclinical status epilepticus in a term infant with meconium aspiration syndrome. The repetitive seizures can be seen as a saw-tooth pattern in the aEEG trend (*top*) and in the band power and spectrogram trends below in this 4-hour recording. The unstable baseline in the aEEG is due to high frequency oscillation ventilation but the seizure activity can clearly be confirmed in the single channel EEG below (duration 25 seconds)

131.3 Conclusions

The EEG, and the aEEG, gives clinically important information on brain function. The EEG/aEEG can diagnose seizures and electrocortical background abnormalities, and the background activity shortly after an insult is highly predictive of neurological outcome. For continuous monitoring, video-EEG is the gold standard, but this method is not available for daily use in most neonatal intensive care units. Other EEG trends such as IBI or frequency-based trends may also prove to be of value in the future. When aEEG/EEG is recorded with a reduced number of electrodes, it is necessary to perform repeated EEGs and perform the monitoring in close collaboration with clinical neurophysiologists or neurologists.

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Neuroimaging Studies

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132.1 Introduction

Many years have passed since the introduction of cranial transfontanellar ultrasound (CUS) to diagnose acquired brain lesions in neonates. CUS remains an important technique in the daily practice of neonatal units but major improvements have been obtained by combining different imaging modalities. Magnetic resonance imaging (MRI) is the modality that allows assessment of the developing brain in great detail because of its resolving power and non-invasiveness. MR techniques are unique in that they provide not only detailed structural but also metabolic and functional information without the use of ionizing radiation. Conventional MRI is therefore now widely used for identifying normal and pathologic brain morphology, and giving objective information about the structure of the neonatal brain during development and injury.

Technical advances in MR have revealed hitherto unknown aspects of brain development and have characterized patterns of injury to the developing brain. Fast diffusion-weighted imaging has provided understanding about brain white matter (WM) connectivity in health and disease. It also allows correlation of the structural development of the brain with the functional development of the child, even in the absence of overt brain lesions. This technique, together with new image postprocessing tools to assess quantitative brain volume and surface changes, have produced more accurate neuroimaging correlates for later neurocognitive disorders.

MR Spectroscopy has also provided new insights into metabolic processes in response to acute and chronic brain injury. Functional MR imaging, although still limited in the newborn, has the potential to throw light on the early functional organization of the brain and the origins of sensory, motor and cognitive functions in the newborn [1, 2].

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132.2 Clinical Use of Neuroimaging Modalities in the Term Newborn

132.2.1 Ultrasound

Initial investigation with CUS in newborn babies born after the 37th week of gestation and presenting neurological symptoms is always justified. The likelihood of CUS showing a perinatally acquired brain lesion in term babies is not high, especially during the acute phase of the disease. CUS can diagnose directly specific pathology like an arterial stroke if the lesion is large, or it can help to identify a brain lesion caused by underlying disease, such as an intraventricular bleed or a posterior fossa hemorrhage caused by traumatic birth or by venous thrombosis. The value of CUS during the acute phase of an hypoxic ischemic encephalopathy is limited since it relies on the exclusion of other lesions which may mimick the clinical features of asphyxia (i.e., malformations, major subarachnoid or subdural hemorrhages, and, more rarely, stroke) (Figs. 132.1, 132.2). CUS is also useful for the identification of severe basal ganglia lesions that become visible only a few days after the asphyxic event.

132.2.2 The Role of MRI in the Symptomatic Term Newborn Baby

Seizure is a striking clinical feature of the neurologically abnormal neonate. It is useful to differentiate fitting babies born with normal Apgar scores from those who are born in a very depressed state because of perinatal asphyxia. The most common causes in the asphyxic group is focal brain injury due to arterial infarction, hemorrhage, and hypoxia-ischemia with typical lesion distribution described in detail below. Neonatal seizures may be caused by central nervous system infections, transient metabolic disturbances like hypoglycemia, congenital abnormalities of the brain including those due to chromosomal

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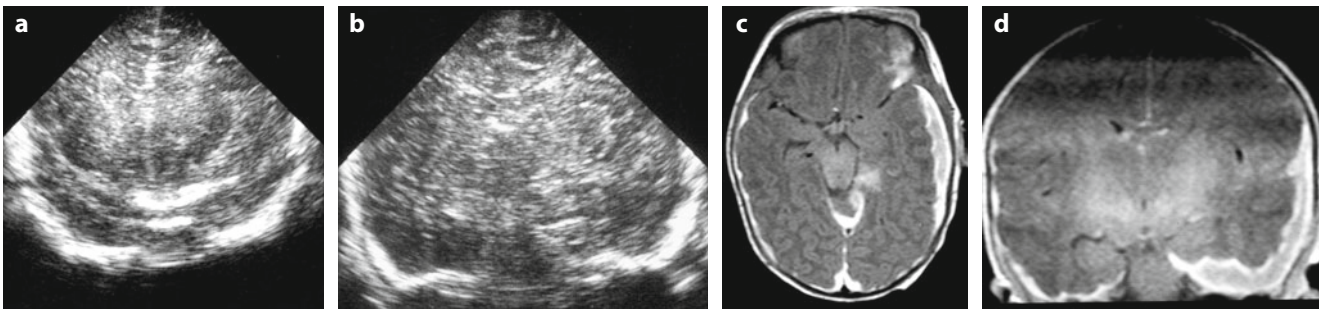

Fig. 132.1 A term baby born after ventouse application and mimicking perinatal asphyxia syndrome, with acidosis at birth and seizures at 6 h of life: CUS coronal scans (**a**, **b**), MR axial T1 (**c**) and coronal T1 (**d**) scans. Neuroimaging was performed at 24 h of life. US showed midline shift and left increased echogenicity, highly suspicious for arterial infarction. MR clearly showed a subarachnoid hemorrhage probably related to dystocia. At 5 years of age the baby was neurogically normal

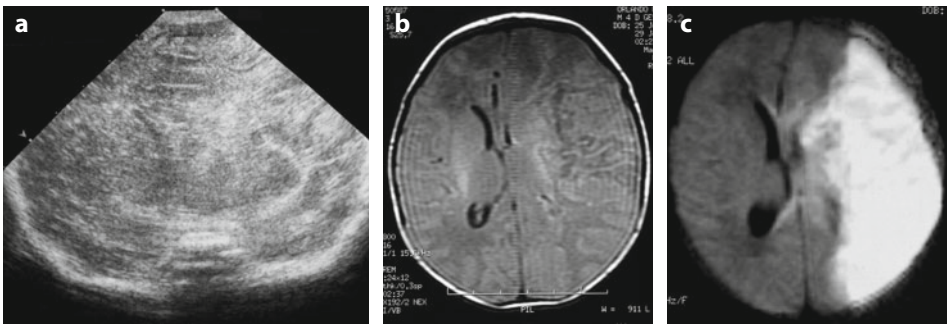

Fig. 132.2 A large perinatal arterial infarct of the left middle cerebral artery is shown on all the scans: **a** US with area of increased echogenicity, **b** conventional MR axial T1 scan with evident midline shift and **c** even better from the huge area of restriction (*white*) at DWI. The baby was macrosomic, born by vaginal delivery and depressed at birth needing resuscitation and mechanical ventilation for a few hours

anomalies, inborn errors of metabolism and benign neonatal convulsions, which may be familial or non-familial, and detailed neuroimaging is an important tool in the diagnostic work-up. Brain malformations range from highly localized focal dysplasia not diagnosable by CUS to catastrophic defects, which are partially diagnosable by CUS.

In all instances of seizures in term newborns, MRI is the ideal diagnostic neuroimaging tool. A clinical diagnosis has to rely on a careful medical history and on consideration of the most likely causes, for instance if seizures occur unexpectedly in a perfectly normal newborn, stroke and hemorrhage have to be the first disorders to be excluded [1, 3].

132.2.3 Neonatal Stroke

An acute neurological syndrome is not essential for the diagnosis of neonatal stroke [4]. The pathogenesis of neonatal stroke is discussed in detail in Chapter 139. Here emphasis is given to the neuroimaging required for the diagnosis of neonatal stroke (Fig. 132.2). As a bedside method, CUS is useful when it shows areas of asymmetrical echogenicity, usually during the first 12–36 hours following the onset of seizures. Nevertheless, MRI is the standard neuroimaging tool for the diagnosis of neonatal stroke. Conventional MRI shows loss of cortico-subcortical differentiation in both T1- and T2-weighted imaging. This is due to increased T2 signal intensity in the edematous cortex, which approaches the signal intensity of the unmyelinated white matter. This is also called the disappeared cortex sign (Fig. 132.3).

The best modality to identify a focal ischemic infarct is diffusion weighted imaging (DWI). DWI shows a striking reduction of water molecule movement (Fig. 132.2), corresponding to a decrease in the apparent diffusion coefficient (ADC) in the acute phase. There is normalization by 6–10 days followed by tissue dissolution, which results in a porencephalic cyst with T2 characteristics of cerebrospinal fluid (CSF). Areas of decreased values can be observed acutely, even a few hours after the beginning of a stroke, when conventional MR imaging remains normal until at least day 4 [5–9]. The cavitational phase of the infarction is recognizable using all techniques, and it occurs at a variable time after the initial insult. The pattern of signal intensity changes seems to be remarkably consistent among patients, suggesting that perinatal arterial ischemic stroke in symptomatic term infants occurs within a very limited timeframe around birth.

The majority of these focal lesions affect term babies and seem to be less common after birth by cesarean section performed in the absence of labor [10]. Premature infants seem

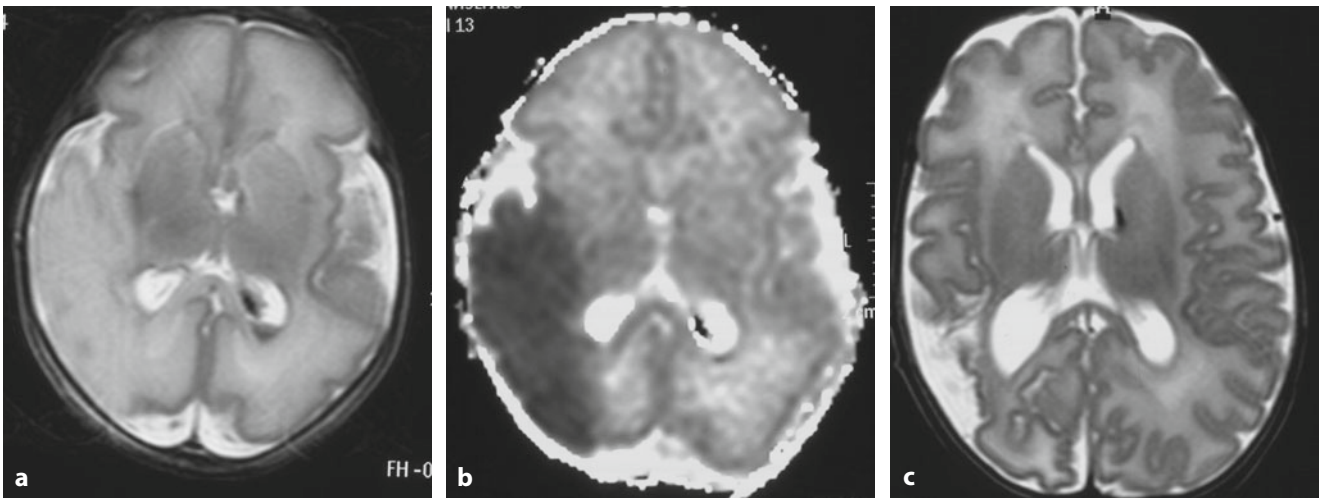

Fig. 132.3 A 30 weeks preterm baby showing at 4 days of life abnormal neuroimaging by MR axial T2 scan (**a**) and MR axial ADC map (**b**), highly suggestive of a right arterial infarct: loss of cortical details on T2, more obvious reduction in apparent diffusion coefficient values. At term-equivalence MR axial T2 scan (**c**) showed that the resulting infarcted area was smaller. At 2 years of age the child was treated for recurrent seizures, but 18 months later was off medications. At 5 years of age, the child presented with a left hemiplegia and good independent walking

to be less vulnerable to this disease, although the incidence has been reported as particularly high in certain studies [11, 12].

132.2.4 Asphyxia

Although antenatal factors have been implicated in the etiology of hypoxic–ischemic encephalopathy (HIE), evidence of antenatal injury is rarely seen on early MRI scans in neonates who present with HIE [3]. MRI remains the best technique to detect perinatally acquired cerebral lesions, and the pattern and severity of the lesions are a reliable guide to prognosis. In the presence of a sentinel event consistent with a severe acute hypoxic–ischemic insult, lesions in the basal ganglia and thalami can be associated with abnormalities in specific cortical regions and in the adjacent subcortical white matter. Abnormal signal intensity in the posterior limb of the internal capsule coexists with lesions in the basal ganglia and thalami (BGT) and is a powerful predictor of abnormal motor outcome (Fig. 132.4). Moderate and severe lesions in the basal ganglia and thalami and severe white matter lesions are associated with cerebral palsy [13]. In approximately 50% of neonates with BGT lesions there will be more extensive WM abnormalities. The motor outcome for these children is still dictated by the BGT lesions, but WM involvement may exacerbate any cognitive deficit. However, infants with severe BGT lesions have severe cognitive impairment regardless of the severity of additional WM involvement. Later there is often white matter atrophy due to the original lesions in the basal ganglia.

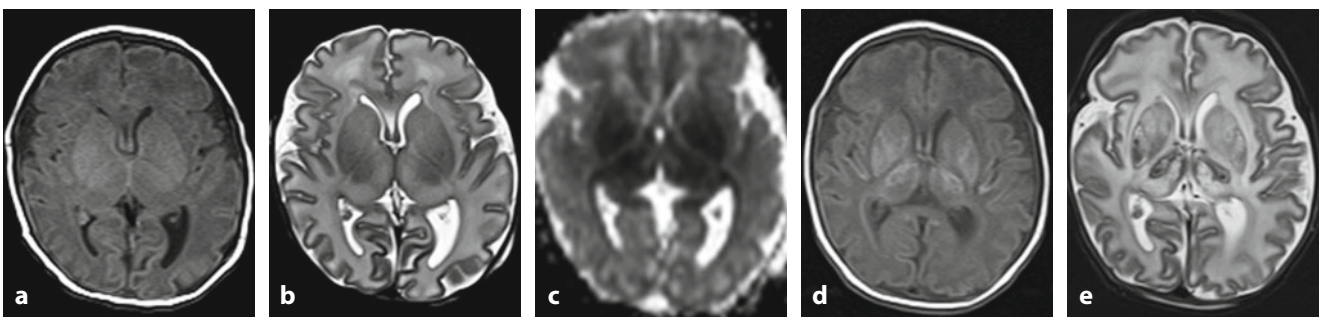

Fig. 132.4 A severe asphyxiated baby born by emergency cesarean section due to maternal rupture of the uterus. **a–c** MR axial T1 and T2 scans and ADC map. There is a markedly abnormal reduction in ADC values at the level of all basal ganglia and thalami on day 4 of life after being treated with hypothermia. **d–e** MR axial T1 and T2 scans. Two weeks later the severity of lesions at the basal ganglia and thalami is obvious. At 15 months, the baby developed severe developmental delay and epilepsy (West syndrome)

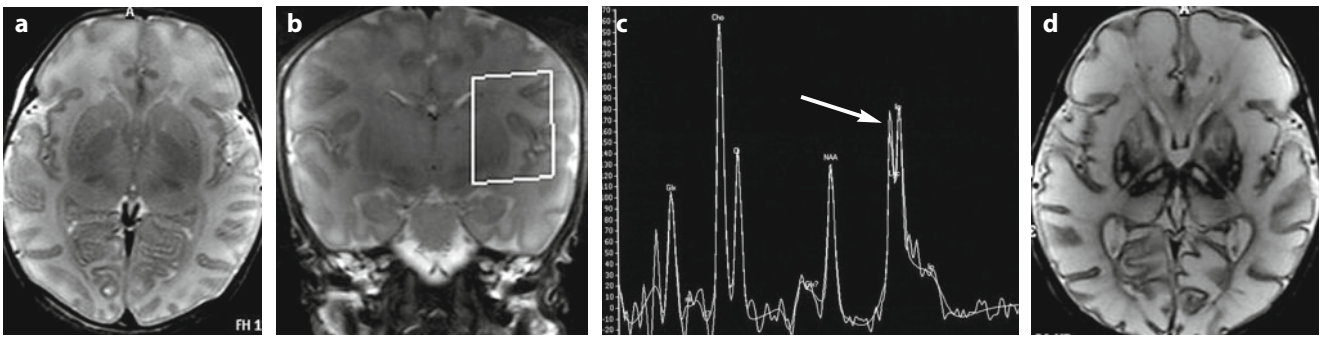

Fig. 132.5 A very severely asphyxiated baby with early scan at 36 h of life. MR axial and coronal T2 scans (**a**, **b**) show uncertain signs at this stage (diffuse increased signal), while MR spectroscopy (**c**) in the region of interest (highlighted) shows a severely abnormal peak of lactate (*arrow*). Three weeks later, an axial T2 scan (**d**) showed dramatic evolution in a pre-multicystic encephalomalacia damage. The baby was born by emergency cesarean section following severe abruptio placentae

DWI seems to underestimate the severity of brain lesions during perinatal asphyxia at early scans, particularly at the level of basal ganglia. Nevertheless, if DWI of the basal ganglia is abnormal, the developing lesions are very likely to be severe (Fig. 132.4).

The ability of MRI to predict neurological outcomes up to 18 months of age in babies who have undergone therapeutic hypothermia is unaffected. In a large cohort of infants who had an MRI after HIE and had previously undergone hypothermia, there were no unusual patterns of lesions and no increase in hemorrhagic or thrombotic lesions. [14]. A common question concerns the optimal timing of an MRI in perinatal asphyxia. The answer relates to the reason for the scan. If it is being done for a differential diagnosis (Fig. 132.1), the scan should be done as soon as possible. On the other hand, if the MRI is being done to predict prognosis, it is better to wait until the 7th day of life, and then to perform a second scan during the second week of life [3]. A repeat scan is particularly useful in the case of an asphyxiated baby born before the 37th week of gestation to look at the posterior limb of internal capsule (PLIC), which is such an important marker of the normal development.

Proton magnetic resonance spectroscopy (^{1}H-MRS) permits the non-invasive study of metabolic alterations in the brain tissue. It has also entered the clinical arena of MR techniques used routinely for the evaluation of the brain. When oxidative phosphorylation is impaired, energy metabolism follows the alternative route of anaerobic glycolysis and produces lactic acid. Lactate has a chemical shift of 1.3 ppm and presents as a doublet peak in the *in vivo* ^{1}H-MRS due to coupling effects. Groenendal et al [15] first described markedly elevated lactate levels in 5 infants with severe perinatal asphyxia. Early spectroscopy (<18h after the event) and measurement of high Lac/Cr ratios in ^{1}H-MRS correlate well with neurodevelopment at 1 year [16]. MRS performed in the first 24h after an insult is sensitive to the presence of hypoxic-ischemic brain injury, and seems to be suitable for the detection of brain injury on the first day when conventional MR imaging and DWI might not yet detect the injury (Fig. 132.5) [17].

132.3 Neurological Symptoms Associated with Neonatal Conditions

Unexpected neurological signs may be due to hypoglycemia [3]. An MRI brain scan is justified in the case of symptomatic hypoglycemia even in the absence of abnormalities at CUS. Infarction following hypoglycemia has been reported in the newborn and specific imaging patterns have been attributed to neonatal hypoglycemic injury [1], e.g. bilateral, fairly symmetrical, evidence of injury to the occipital and posterior parietal cortices and underlying white matter. The cortical ribbon may be lost on T2 as well as T1 MRI, and there may be infarction with subsequent cavitation. Injury beyond the area covered by the posterior cerebral artery is too extensive to be classified as watershed, but the appearance on imaging is similar. The lesion should not be regarded as a stroke (Fig. 132.6) [4]. Another less frequent post-hypoglycemic paradigm is unilateral complete hemispheric neocortical necrosis. The most frequent abnormalities affect occipital white matter and cortex, although there can be other lesions mimicking asphyxia and there is an overlap of symptoms between perinatal asphyxia and hypoglycemia [18].

132.4 Timing of Scanning

Perinatal brain injury leading to brain lesions visualized by MRI evolve over time and are fully developed between 1 and 2 weeks from delivery. By this time, neonates are likely to be clinically stable and off assisted ventilation and MRI can give

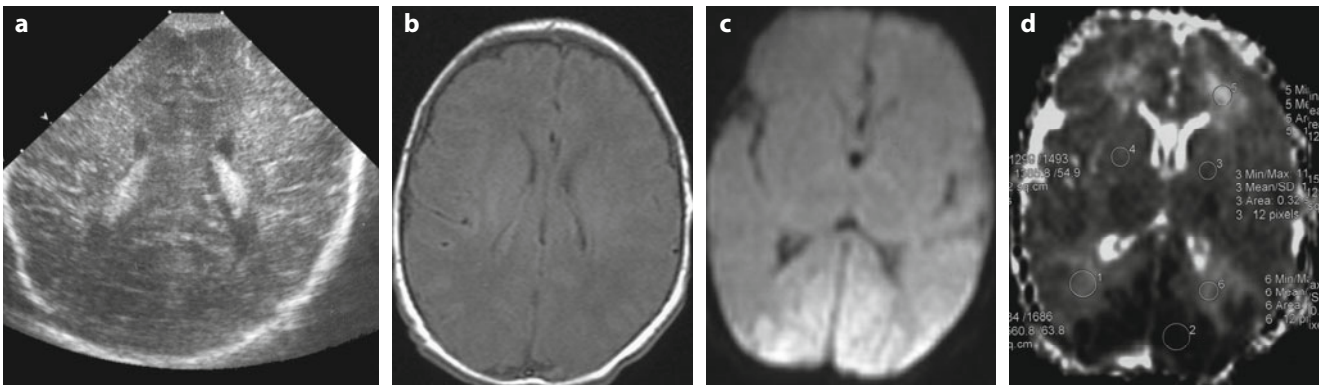

Fig. 132.6 Neuroimaging performed 2 h after the onset of a severe hypoglycaemic event: coronal CUS (**a**) and MR axial T1 (**b**) scans showed no abnormality, which was different to MR axial DWI (**c**) and the axial ADC map (**d**), which showed respectively reduction and pathologically low ADC values in the posterior areas of the brain

an estimation of the extent of lesion load. Although neonatologists may request earlier scanning to make a diagnosis or to assist clinical management, imaging within the first couple of days may show only minor abnormalities in the presence of significant brain injury. Early (<48h from insult) MRI should always include DWI and ^{1}H-MRS if possible. MRS can play an important role in the assessment of encephalopathic term infants. Raised lactate/creatine ratios or absolute concentrations of lactate earlier than 24 h are reliable indicators of cellular injury (Fig. 132.5).

DWI should identify infarcted WM but is not always so reliable at detecting significant injury to the basal ganglia and thalami. Measurement of ADC values and comparison with regional normal values during development are crucial for identifying areas of ongoing injury [5, 19]. As in the case of adult stroke, the DWI visual appearances of infarcted tissue are obvious early and last for approximately 1 week, by which time conventional imaging should be obviously abnormal. Sometimes visual analysis of the DWI is unremarkable or difficult to interpret, even in the presence of severe damage.

Most scanners have the software necessary to obtain ADC from the diffusion images. ADC values should be measured in all infants even when the DWI appears normal. During the first week after injury, ADC values are usually decreased in the presence of ischemic WM, but may be reduced, normal or elevated when there are clinically significant basal ganglia lesions. Difficulties in interpreting DWI findings have led to studies using diffusion tensor techniques to explore tissue microstructure further (see under advanced techniques).

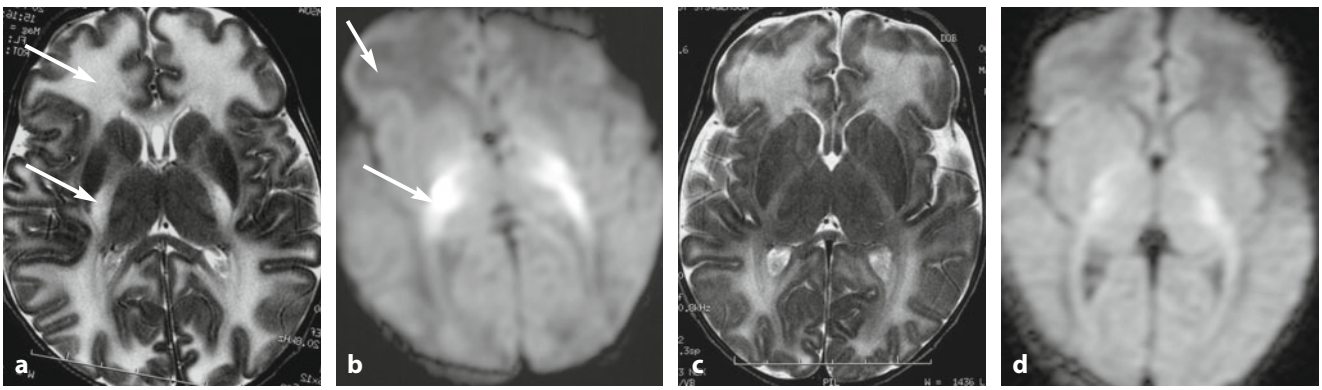

Fig. 132.7 A baby with maple syrup urine disease showing at the first MR (**a**, **b**) severe abnormalities of the signal (*arrows*). On axial T2 scan (**a**) increased signal (*arrows*) in the frontal white matter and at the level of posterior limbs of internal capsule (PLIC) are observed. Interestingly the DWI scan (**b**) shows a completely different pathogenesis; PLIC are represented by a restriction signal on DWI, signs of reduced movements of water molecules, while in the frontal areas DWI shows an opposite signal (increased water molecules movement) very likely expression of vasogenic oedema. **c**, **d** A week after renal dyalisis performed to reduce the amount of the toxic levels of leucine a dramatic improvement of the signal abnormalities is observed

132.5 Interpreting Difficult Cases

132.5.1 Metabolic Disorders

Occasionally infants with an apparent global hypoxic ischemic injury have a metabolic disorder which either masquerades as an acute insult or coexists with one. This is important to recognize because the implications for allegations of medical negligence and for genetic counseling are considerable. Conventional MRI is able to identify brain malformations that may accompany some metabolic disorders, such as callosal agenesis in non-ketotic hyperglycinemia or cortical migration defects in peroxisomal disorders such as Zellweger's syndrome [3]. White matter in these infants may have long T1 and long T2 times, and this may be reflected in increased ADC values. Occasionally the results from DWI are more informative. In infants with maple syrup urine disease DWI is pathognomonic, showing actively myelinating areas (Fig. 132.7) [20]. DWI may also allow differentiation between cytotoxic (reduced ADC) and vasogenic (increased ADC) edema. However, areas showing reduced ADC values may normalize with treatment, and therefore DWI has the potential to be used to monitor therapy. Reversible ADC reduction has also been documented in other metabolic conditions such as hypoglycemia and hypernatremia [21]. A suspicious pattern of diffusion should alert the clinician to the possibility of a metabolic disorder. Abnormalities e.g., affecting the cerebral peduncles and internal capsule, may mimic those found with more straightforward HIE, and an underlying metabolic disorder may be missed. This is particularly likely if a neonate dies in the first few days, as the characteristic clinical evolution of the metabolic disorder has not yet developed. This emphasizes further the need to investigate fully any infant presenting with neonatal seizures, not only by a careful history and examination, but also with metabolic investigations and neuroimaging [3]. The latter should be MRI and include DWI and 1H-MRS if possible. If an infant dies before imaging is possible, postmortem MRI should be considered as an adjunct to autopsy, especially if the parents are unlikely to agree to a conventional postmortem examination.

132.5.2 Sinovenous Thrombosis

The incidence of sinovenous thrombosis is not well known. It has been underestimated for several reasons: many clinicians are unaware of the condition, and the clinical presentation is not specific although it is possible to differentiate between an early presentation within 48 h of birth, and a later one. An early presentation may be confounded by co-morbidities (maternal pre-eclampsia/hypertension, fetal distress) and acute illness (asphyxia, respiratory problems), whereas a later presentation is more often associated with seizures, lethargy, apnea and poor feeding. Acute illness, e.g., dehydration, may also be associated with delayed presentation. Seizures occur in about two-thirds of cases and can be subtle, focal or generalized, irrespective of the time of presentation [22]. The two major goals of radiological diagnosis are imaging of the thrombus and any associated cerebral lesions such as hemorrhage due to venous infarction. An unexplained intraventricular hemorrhage in a term baby or a late-onset intraventricular hemorrhage in a late-preterm baby should raise the suspicion of a sinovenous thrombosis [23]. A typical infarct leaves hemosiderin and gliosis in the residual scar, sometimes with calcification along the vein. There may be evidence of collateral escape. Tissue damage is often overestimated because of the presence of blood that dissects or displaces tissue. Over of the folowing weeks or months, a sinus gradually recanalises. Sinovenous thrombosis in a preterm may present with WM lesions that look like leukomalacia [22]. Color flow Doppler can be useful when there is an inability to visualize a known vein (such as the internal cerebral vein), although it is very difficult to identify a clot in these vessels. The diagnosis by computed tomography (CT) requires administration of intravenous contrast medium highlighting filling defects in the sinuses, and may show classic signs, such as the empty delta sign or the cord sign, whereas CT venography (CTV) provides more accurate evaluation of the venous sinuses. MRI is an excellent tool for imaging the parenchyma, as it requires no ionising radiation or dye, but it is difficult to interpret signals in sinovenous channels. On conventional MRI, the thrombus appears as an increased signal on T1-weighted images and as a decreased signal on T2-weighted images along the vessels

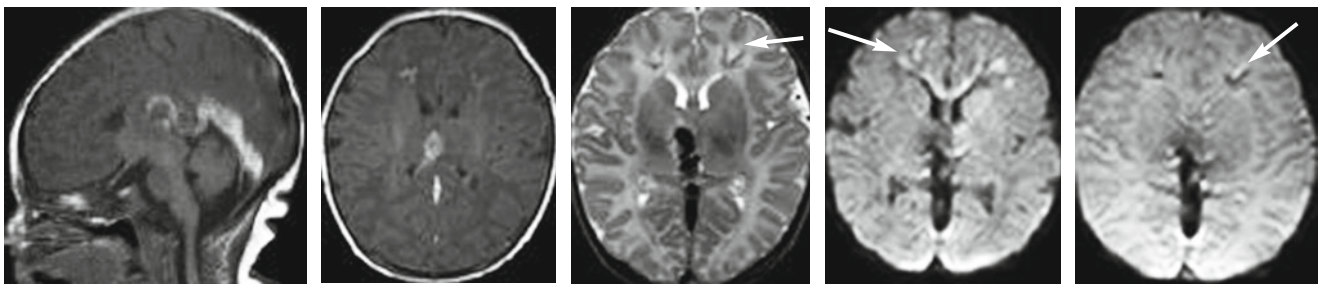

Fig. 132.8 A term baby with an evident and diffuse venous thrombosis, showing thrombi and mild involvement of frontal medullary veins (*arrows*). *Left to right*: MR sagittal T1, axial T1, axial T2, DWI and paramagnetic. (Courtesy of S. Martinelli)

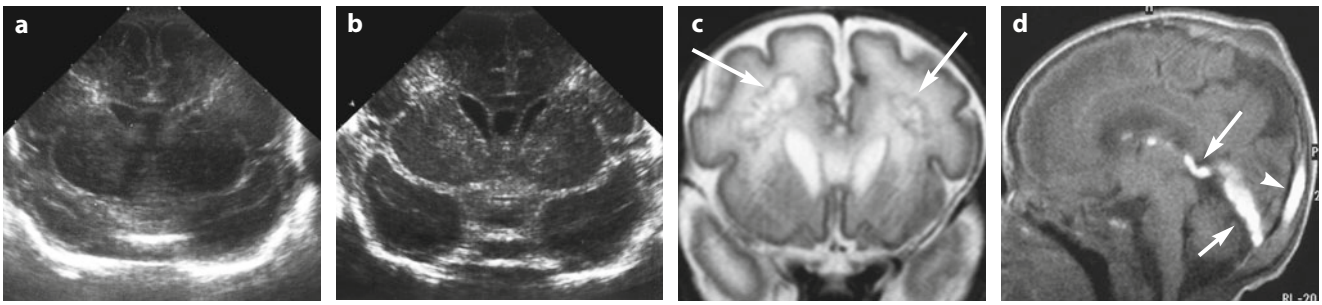

Fig. 132.9 CUS and MR scans at different times in a 35 weeks baby. The first US scan at 4 days of life (**a**) shows normal periventricular echogenicity, progressively increasing at day 9 (**b**). Later MR T2 scans at 3 weeks of age shows: (**c**) cavitations in periventricular area (*arrows*) and (**d**) thrombi in the deep venous system (*arrows*) and partially in the sagittal sinus (*arrowhead*)

involved (Fig. 132.8). Major venous cerebral sinuses are most frequently affected, but there may also be thrombosis in the inner small veins of the deep venous system draining the blood from the germinal matrix and the periventricular WM. A magnetic resonance venogram is an excellent aid, although caution is needed as the slow-flow signal can be misdiagnosed as a no-flow signal. Accordingly, the high proportion of flow gap in the venous sinuses of neonates, particularly the superior sagittal sinus, may be due to the smaller caliber of the venous sinuses, slow flow and skull moulding. The diagnosis of sinovenous thrombosis is further compounded by the fairly common observation of focal bilateral lesions in the brain parenchyma, which may be associated with intraventricular hemorrhage [24]. This strongly suggests sinovenous thrombosis in mildly preterm babies especially when increased periventricular echogenicity follows a normal echogenicity (Fig. 132.9).

132.6 Abnormal Fetal Neuroimaging

With the advent of prenatal neuroimaging, including fetal MRI, it is not uncommon to perform CUS or MRI in neonates to confirm or further investigate abnormalities suspected or diagnosed during fetal life. The most common conditions are idiopathic ventriculomegaly, agenesis of corpus callosum and abnormalities of posterior fossa. A postnatal MRI may be used also to investigate what has been called idiopathic fetal ventriculomegaly that could indicate brain maldevelopment. The MRI examination may rule out aqueduct stenosis or rarer causes of ventricular dilatation like fetal intraventricular hemorrhages. An MRI may be performed in neonates with agenesis of the corpus callosum, mainly to exclude additional cortical abnormalities which a prenatal MRI performed before gyration and sulcation (i.e. before the 25–26th week of gestation) could not definitely exclude.

Although fetal MRI can provide additional information about suspected posterior fossa anomalies, it is important to be aware of its limitations, particularly when performed very early in gestation. There is therefore a need for a follow-up MRI postnatal scan to confirm the posterior fossa abnormality.

132.7 Imaging the Preterm Newborn Brain

Several different aspects have to be considered when considering brain lesions affecting the developing brain of premature babies, i.e. before 37 weeks of gestation and including brains of very different developmental stages and vulnerability for injury. Typical brain lesions less frequently affect the relatively large number of late preterm babies, and are much more frequent in the extremely low birth weight (ELBW) babies, who are fewer in number but very vulnerable [2]. In the late preterm group, brain injury comprises multiple lesions, principally germinal matrix intraventricular hemorrhage, venous hemorrhagic infarction, post-hemorrhagic hydrocephalus, periventricular leukomalacia and cerebellar lesions. However, many preterm infants have neurodevelopmental delay without having been diagnosed with any of these typical perinatal brain injuries. In these babies, abnormal development of the brain is possible even in the absence of common brain lesions (i.e., there may be loss of WM, even in the absence of periventricular leukomalacia). Early exposure to a challenging postnatal environment and removal from a protective milieu are factors that interfere profoundly with normal development, especially in the most vulnerable babies [25, 26].

132.8 Specific Lesions in the Most Premature Babies

132.8.1 Germinal Matrix-Intraventricular Hemorrhage (GMH-IVH)

The lower the gestational age, the higher the risk of developing germinal matrix-intraventricular hemorrhage (GMH-IVH),

which is the most frequent cause of intracranial hemorrhage in the neonate. Ultrasound is now accepted as the primary modality for the diagnosis of GMH-IVH and there have been full descriptions of the ultrasonic findings [27]. GMH is seen as an area of intense increased echogenicity beneath the floor of the lateral ventricle, just anterior to the caudothalamic notch. The abnormality should be seen in two planes, and should be distinguished from other normal echogenic structures, such as the normal choroid plexus. Larger hemorrhages may rupture into the lateral ventricle, and, when acute, appear as an echogenic clot within the ventricle which may distend the ventricular lumen.

The appearance of GMH-IVH and venous infarction change with time (Fig. 132.10). GMHs resolve leaving a small subependymal cyst or a linear echodense line. The margins of a clot within the lateral ventricles remain echogenic, whilst the more central region gradually becomes increasingly echopoor and smaller until the hemorrhage finally resolves by approximately 12 weeks of age.

Several grading systems have been proposed to classify GMH-IVH. The majority are based on the extent of bleeding into the germinal matrix and lateral ventricle, the development of venous infarction and the presence of ventricular dilatation.

The accuracy of ultrasound imaging in the diagnosis of GMH-IVH is reported to be approximately 90%, which is comparable to diagnosis by CT [27]. Thus ultrasound imaging has now become the imaging modality of choice for the detection of GMH-IVH. It can also be performed on a daily basis if necessary, with no risk of exposure to ionizing radiation or the need to move the infant to the imaging department. A wider use of MRI on premature babies has shown excellent visualization of the germinal matrix (GM) with high signal on T1- and low signal on T2- weighted images, especially up to the 30th week of gestation. After this time, T2-weighted images remain the best sequence to follow the physiological GM involution, which occurs with increasing gestational age. Germinal matrix hemorrhage has similar characteristics to normal germinal matrix, but is detectable due to its irregular shape and asymmetry. Very preterm babies may show small lesions consistent with subependymal hemorrhage in areas other than the classic site of the caudo-thalamic notch, most often in the posterior horns. These hemorrhages seem not to be visible on ultrasound [28]. Intraventricular hemorrhage, more often identified in the posterior horns of the lateral ventricle, is an obvious diagnosis with MRI, making this technique the most accurate for GMH-IVH investigation, although MRI scanning is not practical for sick unstable neonates during the first days of life. MRI has improved the detection of venous infarction associated with GMH-IVH, although caution is needed because subependymal hemorrhages may appear to have white matter involvement due to partial volume effects. Keeney demonstrated that an MRI performed between 29 and 44 weeks' postmenstrual age was superior to both US and CT in assessing the extent of any parenchymal injury associated with GMH-IVH [29]. MRI signal shows changes following any form of intracranial hemorrhage, including IVH, according to the timing of hemorrhage. The typical hyperintense intraventricular hemorrhagic signal on T1 usually appears in the so-called subacute phase, usually between 4 days and 2 weeks after the initial bleed, while the long-lasting hemosiderin deposition seems to be visible only after 3–4 weeks [1].

132.8.2 Parenchymal Hemorrhage/Venous Infarction

In general, ultrasound scanning should be the first imaging modality, but if the ultrasound scan is non-conclusive and there is a strong clinical suspicion of acute hemorrhage, an MRI should be considered. Hemorrhage occurring peripherally in the brain or in the cerebellum may not be easy to visualize by ultrasound, particularly if the anterior fontanelle is relatively small. MRI scanning is the most sensitive technique and should be substituted for CT at all times.

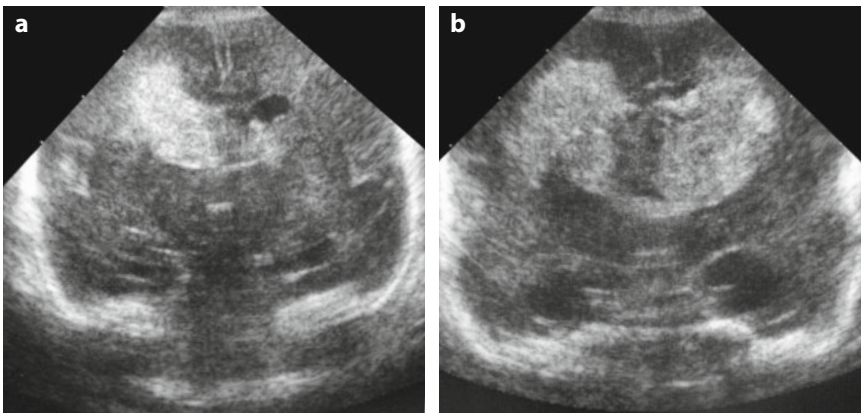

Fig. 132.10 CUS Coronal scans. **a** A 24 weeks baby scanned at 8 h of life and showing severe intraventricular hemorrhage on the right with adjacent venous infarct. **b** The same baby scanned 12 h later showing a further deterioration with a bilateral intraventricular hemorrhage and very rare double venous infarct. The baby died aged 36 h

GMH-IVH can present with unilateral parenchymal hemorrhage, better known as venous infarction at the first scan, or more commonly within a few hours in a later scan. The region involved can be quite large and just dorsal and lateral to the external angle of the lateral ventricle, usually sparing the cortical mantle, although location and size can vary [27]. Less often, the lesion develops in more posterior parts of the brain, in the temporal lobe or around the atrium. In these cases, the inferior ventricular veins or lateral atrial veins are involved. It is still possible to have bilateral venous infarction, but this condition is extremely rare and well differentiated from PVL. At first, the lesion appears as a triangular density often not touching the ventricle, but later the lesion grows and extends to the ventricle, merging the area of increased density due to matrix hemorrhage [27]. Sometimes there is no progression to this stage and the lesion remains as a triangular density. The hyperdense area tends to decrease in size during the second week and the real infarcted area bends up being smaller than expected. Cystic degeneration is the most common evolution of severe cases with a smooth-walled cavity in the parenchyma communicating with the ventricle (unlike in periventricular leukomalacia) [27].

132.8.3 Hydrocephalus

Ultrasound imaging is the most appropriate modality for the initial assessment of ventricular size. The slightly rounded shape of the frontal horns can represent the initial appearance of dilatation while balloon-shaped frontal horns are a sign of severe dilatation. Latero-lateral and diagonal measurements of the diameter are a well-established modality by which to monitor ventricular dilatation. Absence of widening of the frontal horns may be falsely reassuring as neonates tend to have dilated occipital horns (colpocephaly). Many units have their own guidelines for measuring frontal horns, but very often not for measuring the posterior horns [27].

MRI can be useful as detailed imaging is often required prior to shunting and also after surgery to verify the functioning of the shunt, provided the intraventricular device is MRI compatible .

132.8.4 Pseudocysts

Cavitation in the germinal matrix represents a phenomenon called germinolysis. There is a lack of proper epithelium and it occurs typically at the caudo-thalamic notch, causing a pseudocyst [30]. During the postnatal period, pseudocysts occur mainly following small to moderate GMH-IVH, although a mechanism based on pure infarction of the germinal matrix has been hypothesized. Pseudocysts are sometimes detected at the caudo-thalamic notch a few weeks after birth in normal preterm babies with no obvious reason [31].

132.8.5 Cerebellar Hemorrhages

Few *in vivo* studies have focused on cerebellar hemorrhagic lesions. Cerebellar hemorrhages (CH) are known to be highly represented in post mortem studies, varying from 10 to 25% in very low birth weight infants. However, an *in vivo* diagnosis, even with MRI, seems to be around only 10% in preterm infants less than 32 weeks of gestation [32]. Ultrasound studies performed through the posterior fontanelle, which is more sensitive for visualizing the cerebellum than the more conventional view through the anterior fontanelle, show a low incidence of about 3%, especially in extremely low birth weight babies. Ultrasound using the occipital window appears to be the best approach and the incidence is probably higher [1]. In some cases, cerebellar hemorrhages can be severe and associated with flattening of the pons (Fig. 132.11). A degree of pontine atrophy has been observed in preterm babies with PVL, and a flattened anterior curvature of the pons has been detected by MRI in 28 premature infants born before the 30th week of gestation [32].

Acquired pontocerebellar atrophy should be differentiated from congenital pontocerebellar hypoplasia (an association of pontine, vermis and cerebellar hemisphere hypoplasia) and marks a neurodegenerative disorder. Acquired forms of pontocerebellar atrophy have been described in association with fetal drug exposure, sepsis in premature babies, twin-to-twin transfusion syndrome and vascular undersupply.

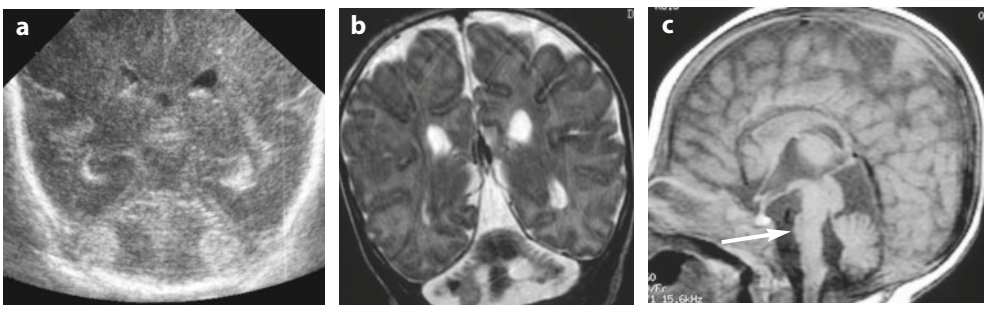

Fig. 132.11 Coronal CUS scan (**a**) of a 24 weeks baby scanned at 10 days and showing bilateral cerebellar hemorrhages. MR scans at term-equivalence: coronal T2 (**b**) showing cerebellar atrophy at the hemispheres; axial T1 (**c**) showing flattening of the pons (*arrow*)

The causes of the association between impaired pontine development and cerebellar hemorrhage remain unknown. The cerebellum receives excitatory input from the frontoparietal cortex via the corticopontocerebellar tracts; an interruption, with secondary degeneration of corticopontocerebellar tracts, could explain pontine atrophy linked to supratentorial lesions, which has been reported in premature babies with little or no PVL. In the same way, primary cerebellar lesions can cause degeneration of pontine fibers through the presence of pontocerebellar tracts. However, a sequence beginning with cerebellar hemorrhage and ending with pontine atrophy has not been demonstrated. The diagnosis of pontine flattening is often made by MRI at term-equivalence. This intrinsic vulnerability of the myelinating posterior fossa at low gestation may partly explain impaired development of the pons.

Clinical follow-up studies are needed to investigate whether or not pontine lesions worsen the outcome of babies with isolated cerebellar lesions.

132.9 Premature Babies: the Importance of White Matter

Periventricular leukomalacia (PVL) is described in detail in Chapters 135 and 136. In clinical practice, at least in the older preterm infant, a white matter disease is ascertained by comparing its echogenicity with that of the choroid plexus. Generally the echogenicity found in early periventricular leukomalacia is similar in intensity to that of the choroid plexus, usually bilateral, slightly asymmetric, sharply delineated, sometimes with nodular components. This has to be differentiated from normal peritrigonal flaring which is perfectly symmetric and has a radial appearance. The evolution of such hyperechogenecity can result in either complete disappearance or its evolution into cysts and/or ventricular dilatation [27]. Cyst formation typically occurs after the second week (10–40 days) after the insult. DeVries et al [33] postulated an ultrasound-based classification for PVL of four grades, increasing grades being associated with increasing neurodevelopmental handicap (Table 132.1). Grade I is transient (> 7 days) periventricular densities without cyst formation. Grade II is when cysts develop but are few in number or localized primarily to the frontal and frontoparietal white matter. When cysts are widespread and extend into the parieto-occipital region they are referred to as Grade III; they may grow and gradually disappear leaving an irregularly dilated lateral ventricle. If cysts are present all the way into the subcortical area resembling porencephaly, this is referred to as Grade IV.

Table 132.1 Ultrasound classification of PVL [33]

Grade I	Transient periventricular echodensities (PVE) (> 7 days)
Grade II	PVE evolving into localized fronto-parietal cystic lesions
Grade III	PVE evolving into extensive periventricular cystic lesions
Grade IV	Echodensities evolving into extensive periventricular and subcortical cysts

Classification requires longitudinal assessment with daily to weekly ultrasound scans.

The predilection of injury to the periventricular white matter is shared by the newer WM diseases identified by MRI *in vivo* studies. White matter injury in preterm neonates is accompanied by diffuse neuronal and axonal disease, affecting not only cerebral white matter but also deep gray, cortex and cerebellum. The vulnerability of the periventricular WM has been extensively studied in the context of PVL and is thought to be due to a combination of the presence of a vascular watershed and an inherent susceptibility of preoligodendrocytes to injury [26]. In addition, there are populations of resident microglia at these sites of periventricular white matter [34]. It is possible that these microglia, which are essential for axonal guidance and white matter tract modeling, are activated abnormally in the presence of injury. In infants with PVL it is recognized that the brain may show a diffuse component around and at a distance from the focal cystic lesions [35]. This is confirmed by *in vivo* imaging where there may be both cystic and chronic changes in diffusion parameters in the white matter of preterm infants who have white matter injury [36].

MR imaging of chronic white matter injury in the immature brain is characterized by the presence of cysts, and, more importantly, by a persistent high MR signal intensity of the white matter in T2-weighted images (DEHSI), which represents diffuse white matter injury or punctuate T1-weighted hyperintensities in the periventricular white matter. PVL is a pathological term that does not imply a particular etiology. Punctate lesions and DEHSI are imaging terms and therefore do not imply either etiology or pathology. Whilst there are many animal models of PVL like lesions, there is as yet no model that has produced an injury that is instantly recognisable as punctate lesions or DEHSI. To confirm such correlations we need either imaging studies of the animal model or human imaging and histological correlations. The latter is difficult because neonates with these milder forms of WM disease are unlikely to die. Attempts to obtain histological correlations would be easier if a diagnosis of punctate lesions and DEHSI could be made routinely using ultrasound [37–39].

The use of MRI as a routine imaging tool in the neonatal intensive care is limited. Florid punctate lesions are detectable by ultrasound, but may be missed, and there is not as yet an ultrasound correlate for DEHSI. *In vivo* imaging of the brain of an animal model has shown an increase in ADC values, as found in both DEHSI and diffuse PVL [40], and at histology this white matter contained increased numbers of activated microglia. It has often been assumed that the short T1, short T2 punctate lesions represent hemorrhage. Against this interpretation is the observation that the signal intensity on T1-weighted images is usually more pronounced than on T2-weighted images and not enhanced by gradient echo imaging. In one attempt at discovering the significance of these MR appearances, these punctate lesions were found

to represent clusters of activated microglia and macrophages containing lipid droplets.

Early assessment of periventricular white matter in preterm infants by DWI shows bilateral periventricular diffusion restriction, which has a distribution that is similar to the typical distribution of PVL when ultrasound and conventional MRI show no or non-specific abnormalities [41]. A reduced ADC in an otherwise normal preterm brain is considered an early indicator of white matter damage (just as a reduced ADC is seen shortly after the onset of an acute cerebral ischemic lesion in the full-term newborn) [42].

132.10 White Matter of Premature Babies at Term Corrected Age

Brain maturation is very rapid during the third trimester of pregnancy and for the preterm infant in the early weeks of life prior to term age. Assessment of brain maturation at term-equivalent age has therefore become an important indicator of brain development with implications for future functional outcome. When preterm infants are imaged at term-equivalent age, the secondary effects of white matter disease on various brain structures can be visualized and quantified. Infants with PVL characteristically show thalamic atrophy and abnormally delayed myelination within the PLIC [43]. These findings can be used to determine whether the child will walk independently. Quantitative studies have also demonstrated a reduced cortical volume at term-equivalent age in infants with white matter injury [42], reduced cerebellar volume [44] and reduced central gray matter volume in premature infants with respiratory problems [45]. Infants with punctate lesions usually show milder abnormalities (Fig. 132.12). A study at term-equivalent age showed that infants with punctuate lesions had reduced myelin and decreased cortical folding [46]. Infants with fewer lesions may show relatively normal imaging. By definition, DEHSI is diagnosed at term-equivalent age as regions of long T1 and long T2 within the white matter. The diffuse T2 hyperintensities or DEHSI, considered to be indicators of the chronic phase of white matter injury, are associated with higher ADC values, confirming a higher local tissue water content and loss of microstructure impeding water diffusion in those areas. Quantitative measures of diffusion at term showed that when compared to preterm infants without white matter injury, premature infants with perinatal white matter lesions had lower anisotropy values (a value that measures directionality of water diffusivity and an expression of fiber density) in the area of previous injury, i.e., the central periventricular white matter, and also in the underlying posterior limb of internal capsule. On quantitative analysis, DEHSI is associated with a reduction in central gray matter volume at term age [47].

Diffusion weighted imaging with diffusion tensor analysis (DTI) has provided new insights into the microstructural white matter development and seems to be an ideal tool to assess alteration of white matter pathways and its consequences for later motor development. Evaluating DTI studies at term-equivalent age with assessments at 18–24 months corrected age (CA), Arzoumanian [48] found that fractional anisotropy (FA) values in the right PLIC were significantly lower for preterms with cerebral palsy than for those with normal examinations. Correlations of DTI values and visual outcome have also been performed [49].

Several authors have investigated the influence of preterm birth on primary cortical folding [50] because of the potential of underlying white matter connectivity to determine gyrification in developing brain.

Functional MRI connectivity (fcMRI), which assesses both neural processing and resting state connectivity, has been less well studied in the preterm population, but may also offer insights into the microstructural mechanisms that support brain development [51]. As with term controls, neonates were found to have resting-state networks that encompassed the

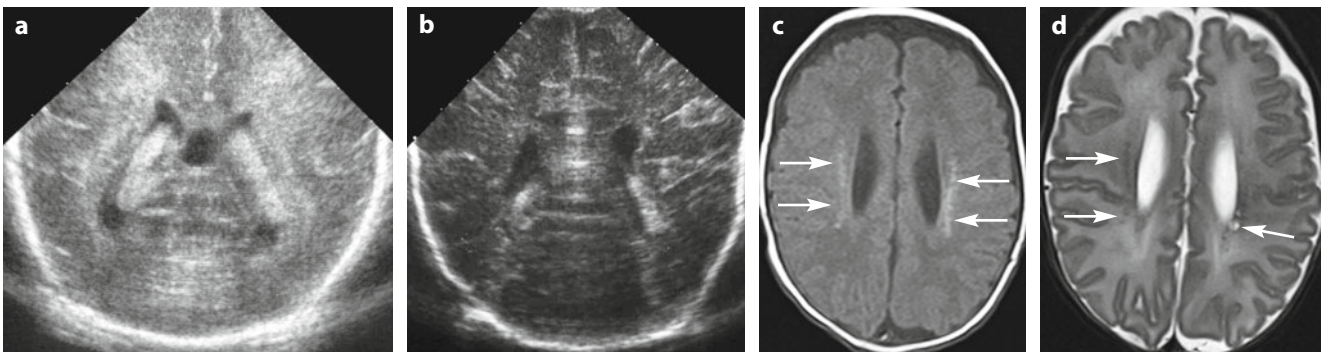

Fig. 132.12 **a** Coronal CUS scan showing a transient increased periventricular echogenicity at 10 days of a 29 weeks baby. **b** CUS scan at term-equivalence showing normal echogenicity, while MR axial scans (**c** T1, **d** T2) highlight periventricular abnormalities (*white arrows*) usually called punctate lesions. On T1 weighted images, these abnormalities at term-equivalence may also represent mild glial scars

primary visual cortex, bilateral sensorimotor areas, bilateral auditory cortex, a network including the precuneus, lateral parietal cortex and the cerebellum and an anterior region that incorporated medial and dorsolateral prefrontal cortex.

Imaging strategies that assess both connectivity and cortical development may provide important early biomarkers predictive of later development. Two relatively new technologies, DTI and fcMRI, combined with advanced image analysis tools such as voxel-based morphometry (VBM) and mathematical morphology-based analysis of cortical folding, provide complementary data for understanding development, injury and recovery in the developing brain. In a recent review Ment et al summarize studies using those new neuroimaging tools [25].

It is generally believed that neurological sequelae are based on specific patterns of nosologically identified brain lesions as an early bedside detection of the major cerebral pathologies, such as GMH-IVH, venous infarction/hemorrhagic parenchymal infarction (PVH), posthemorrhagic ventricular dilatation (PHVD) and cystic periventricular leukomalacia. All these conditions predict of severe neurological impairment. However, the lack of recognizable lesions is not a guarantee for a good neurological outcome, especially for extremely low birth weight babies. In this group of babies, the incidence of lesions which cannot be visualized on CUS, such as those in the posterior fossa, may be underestimated.

The use of CUS has been widely validated in neonatal intensive care units during the last 25–30 years to diagnose intraventricular hemorrhage with its complications (venous infarct, posthemorragic dilatation). More recently, MRI allows assessment of different degrees of white matter abnormalities affecting the brain of premature infants. The extent to which these new techniques are early biomarkers of cognitive outcome in the premature infants needs to be determined, although a recent review appeared to support this view, especially when diffusion tensor techniques were used.

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133

Malformations of Cortical Development: Genetic Aspects

Renzo Guerrini and Elena Parrini

133.1 Introduction

The development of the human cerebral cortex is a complex dynamic process that occurs during several gestational weeks [1]. During the first stage, stem cells proliferate and differentiate into young neurons or glial cells deep in the forebrain in the ventricular and subventricular zones lining the cerebral cavity. During the second stage, cortical neurons migrate away from their place of origin: most cells migrate along the radial glial fibres from the periventricular region towards the pial surface, where each successive generation passes one another and settles in an inside-out pattern within the cortical plate. When neurons reach their destination, they stop migrating and order themselves into specific "architectonic" patterns guiding cells to the correct location in the cerebral cortex. This third phase involves final organization within the typical six layers of cortex, associated with synaptogenesis and apoptosis.

Abnormal cortical development is increasingly recognized as a cause of developmental disabilities and epilepsy. This recognition is due, in part, to the improved use of magnetic resonance imaging (MRI), which makes it possible to assess the distribution and depth of cortical sulci, cortical thickness, the boundaries between gray and white matter, and variations in signal intensity.

Abnormalities of any or all of these features may be observed in different malformations of cortical development (MCD), which may be restricted to discrete cortical areas or may, alternatively, be diffuse [2].

In the following sections, the most frequent causes of MCD will be discussed (Table 133.1).

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133.2 Lissencephaly and Subcortical Band Heterotopia

Lissencephaly (LIS) is characterized by absent (agyria) or decreased (pachygyria) convolutions, cortical thickening and a smooth cerebral surface [3]. Several types of LIS have been recognized. The most common, classical LIS, features a very thick cortex (10–20 mm *vs* the normal 4 mm) and no other major brain malformations.

Subcortical band heterotopia (SBH) is a related disorder in which bands of gray matter are interposed in the white matter between the cortex and the lateral ventricles [3]. Histopathology demonstrates that heterotopic neurons settle close to the "true" cortex in a pattern suggestive of laminar organization.

Three rarer forms of LIS-pachygyria have been identified in recent years [4]. One form, due to mutations of the *TUBA1A* gene, exhibits characteristics that partially overlap with the LIS-SBH spectrum but is often accompanied by cerebellar hypoplasia. A second form, X-linked LIS with absent corpus callosum and ambiguous genitalia (XLAG), results from mutations of the *ARX* gene. A third, recessive form, results from homozygous mutations of the *RELN* gene. LIS due to *ARX* and *RELN* mutations exhibits particular features, which set them out of the classical LIS spectrum.

133.2.1 Genetic Basis and Diagnosis

Two major genes have been associated with classical LIS and SBH. The *LIS1* gene is responsible for the autosomal form of LIS [5], while the *DCX* gene is X-linked [6]. Although either gene can result in either LIS or SBH, most cases of classical LIS are due to deletions or mutations of *LIS1* [7], whereas most cases of SBH are due to mutations of *DCX* [6]. *LIS1*-related LIS is more severe in the posterior brain regions (posterior > anterior or p > a gradient) (Fig. 133.1a), whereas

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 133.1 Genes and chromosomal loci associated with MCD

Cortical malformation	Pattern of inheritance	Gene	Locus	OMIM
Lissencephaly (LIS)				
MDS	AD	*LIS1*	17p13.3	*601545
ILS or SBH	AD	*LIS1*	17p13.3	*601545
ILS or SBH	X-linked	*DCX*	Xq22.3-q23	*300121
ILS or SBH	AD	*TUBA1A*	12q12-q14	*602529
XLAG	X-linked	*ARX*	Xp22.13	*300382
LIS cerebellar hypoplasia	AD	*RELN*	7q22	*600514
Periventricular nodular heterotopia (PNH)				
Classical bilateral PNH	X-linked	*FLNA*	Xq28	*300017
Ehlers-Danlos syndrome and PNH	X-linked	*FLNA*	Xq28	*300017
Facial dysmorphisms, severe constipation and PNH	X-linked	*FLNA*	Xq28	*300017
Fragile-X syndrome and PNH	X-linked	*FMR1*	Xq27.3	*309550
PNH with limb abnormalities (limb reduction abnormality or syndactyly)	X-linked	…	Xq28	
Williams syndrome and PNH	AD	…	7q11.23	
PH	AD	…	5p15.1	
PH	AD	…	5p15.33	
Agenesis of the corpus callosum, polymicrogyria and PNH	AD	…	6q26-qter	
PH	AD		4p15	
PH	AD	…	5q14.3-q15	
Agenesis of the corpus callosum and PNH	AD	…	1p36.22-pter	
Microcephaly and PNH	AR	*ARFGEF2*	20q13.13	*605371
Donnai-Barrow syndrome and PNH	AR	*LRP2*	2q24-q31	*600073
Polymicrogyria (PMG)				
Bilateral frontoparietal PMG	AR	*GPR56*	16q13	*604110
Asymmetric PMG	AD	*TUBB2B*	6p25.2	*612850
PMG and rolandic seizures, oromotor dyspraxia	X-linked	*SRPX2*	Xq21.33-q23	*300642
PMG and Agenesis of the corpus callosum (ACC), microcephaly	AD	*TBR2*	3p21.3-p21.2	*604615
PMG and aniridia	AD	*PAX6*	11p13	*607108
PMG	AD	…	1p36.3-pter	
PMG and microcephaly	AD	…	1q44-qter	
PMG and facial dysmorphisms	AD	…	2p16.1-p23	
PMG and microcephaly, hydrocephalus	AD		4q21-q22	
PMG	AD	…	21q2	
PMG and Di George syndrome	AD	…	22q11.2	
PMG and Warburg Micro syndrome	AR	*RAB3GAP1*	2q21.3	*602536
PMG and Goldberg-Shprintzen syndrome	AR	*KIAA1279*	10q21.3	*609367

AD Autosomal dominant, *AR* autosomal recessive, *PH* periventricular heterotopia.

DCX-related LIS is more severe in the anterior brain (anterior > posterior or a > p gradient) (Fig. 133.1b).

About 60% of patients with p>a isolated LIS (ILS) carry genomic alterations or mutations involving *LIS1* [7]. A simplified gyral pattern in the posterior brain with underlying SBH has been associated with mosaic mutations of *LIS1* [4]. Miller-Dieker syndrome (MDS) is caused by deletion of *LIS1* and contiguous genes and features severe p>a LIS, accompanied by distinct dysmorphic facial features and additional malformations (Fig. 133.1c) [5].

Most *DCX* mutations cause a>p SBH/pachygyria. Mutations of *DCX* have been found in all reported pedigrees and in 80% of sporadic females and 25% of sporadic males with SBH [6]. Genomic deletions of *DCX* are rarely observed [4]. Maternal germline or mosaic *DCX* mutations may occur in about 10% of cases of either SBH or X-linked LIS (XLIS) [8]. Hemizygous males with *DCX* mutations have classical LIS (Fig.133.1 d), but rare boys with missense mutations and SBH have been described [9].

133.2.2 Phenotype

Classical LIS is rare, with a prevalence of about 12 per million births. Patients with severe LIS have early developmental delay, early diffuse hypotonia, later spastic quadriplegia, and eventual severe or profound mental retardation. Seizures occur in over 90% of LIS children, with onset before 6 months in about 75% of cases. About 80% of children have infantile spasms, although electroencephalography (EEG) does not show typical hypsarrhythmia [4]. Most LIS children

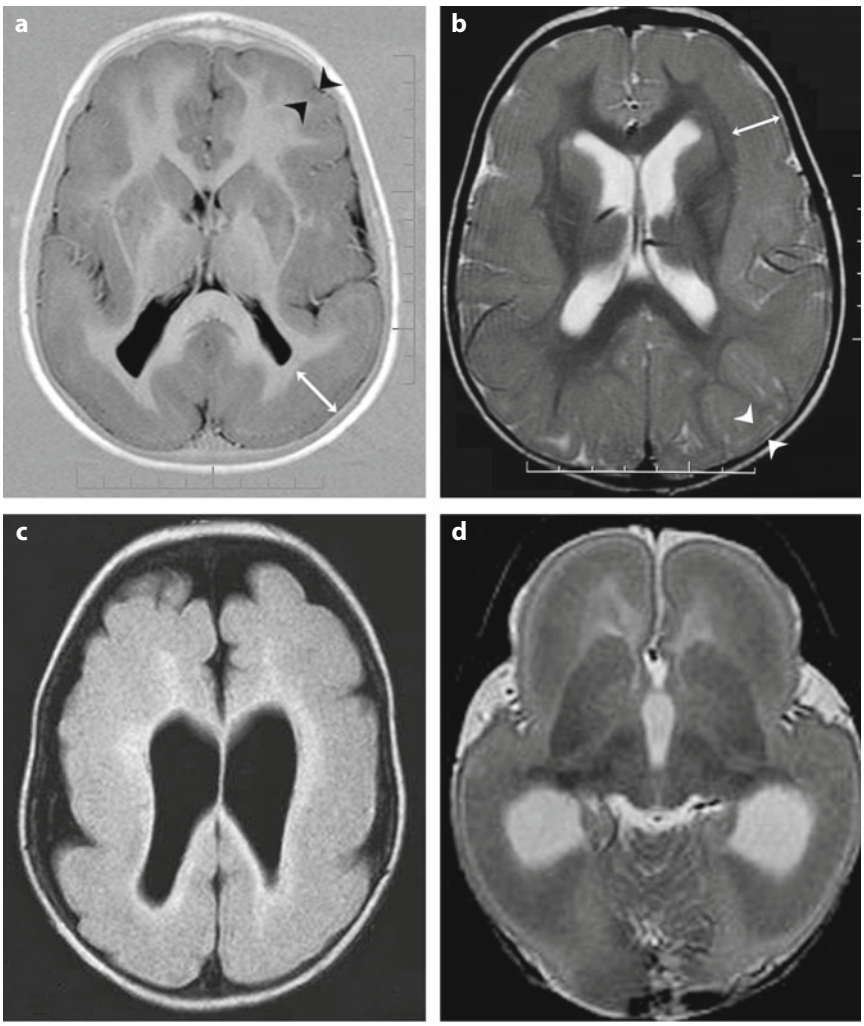

Fig. 133.1 Brain MRI of four different patients; axial sections. **a** classical LIS in a boy with *LIS1* gene mutation; **b** LIS in a girl with *DCX* mutation. In **a**, there is a p>a gradient, cortical thickness is around 6 mm in the frontal lobes (*two black arrowheads*) and around 3 cm in the posterior brain (*white arrow*). In **b** there is a typical a>p pattern; cortical thickness is around 2 cm in the frontal lobes (*white arrow*) and around 4 mm in the posterior brain (*two white arrowheads*). **c** LIS in a patient with MDS. **d** Severe diffuse LIS with relatively small frontal lobes in a boy with *DCX* mutation

subsequently have multiple seizure types. In patients with MDS, classical LIS is accompanied by distinct dysmorphic facial features [5]. The main clinical manifestations of SBH are mental retardation and epilepsy. Epilepsy is present in almost all patients and is intractable in about 65% of cases. About 50% of these epilepsy patients have focal seizures, and the remaining 50% have generalized epilepsy, often within the spectrum of Lennox-Gastaut syndrome.

133.2.3 Laboratory Investigations

In patients with classical LIS, the cytogenetic and molecular investigations are part of the diagnostic process. When MDS is suspected, a standard karyotype and FISH for the 17pl3.3 region is indicated. When isolated LIS is diagnosed, careful assessment of the antero-posterior gradient of cortical pattern abnormality will be suggestive of the involvement of either the *LIS1* or the *DCX* gene.

When LIS is more severe posteriorly, it is worth performing first multiplex ligation-dependent probe amplification (MLPA) in order to rule out *LIS1* deletions/duplications. If a deletion/duplication is not found, *LIS1* sequencing should then be performed. In boys, whose MRI shows more severe pachygyria in the frontal lobes, sequencing of the *DCX* gene is indicated. In patients with SBH direct sequencing of *DCX* should be performed. If a *DCX* mutation is not found, MLPA analysis should then be performed. Direct sequencing is also indicated in the mothers of patients harbouring a *DCX* mutation or other female relatives.

133.2.4 Genetic Counselling

All reported *LIS1* alterations are *de novo*. Given the theoretical risk of germline mosaicism in either parent (which has never been demonstrated for *LIS1*), a couple with a child with lissencephaly is usually given a 1% recurrence risk.

When a *DCX* mutation is found in a boy with LIS, mutation analysis of *DCX* should be extended to the proband's mother, even if her brain MRI is normal. If the mother is a mutation carrier, the mutation will be transmitted according to Mendelian inheritance. If the mother is not a carrier, she can still be at risk of germline mosaicism; the risk of transmitting the mutation may roughly be estimated at around 5%.

133.3 Heterotopia

There are three main groups of heterotopia: periventricular (usually nodular: PNH), subcortical (SBH) and leptomeningeal, of which only the first two can be detected by imaging. PNH is by far the most frequent. SBH is a mild form of LIS and classified in that group.

133.3.1 Periventricular Nodular Heterotopia

Periventricular nodular heterotopia (PNH) consists of nodules of gray matter located along the lateral ventricles with a total failure of migration of some neurons [3]. It ranges from isolated, single, to confluent bilateral nodules (Fig. 133.2). The overlying cortex may show an abnormal organization.

133.3.1.1 Genetic Basis and Diagnosis

PNH is a clinically and genetically heterogeneous disorder occurring most frequently in women as an X-linked trait (classical bilateral PNH), associated with high rates of prenatal lethality in male fetuses, and 50% recurrence risk in the female offspring. Almost 100% of families and 26% of sporadic patients, harbor mutations of the *FLNA* gene [10], which also causes cardiovascular abnormalities in some patients of both sexes and gut malformations in boys. Only a few living male patients with PNH due to *FLNA* mutations have been reported [11]. A rare recessive form of PNH owing to mutations of the *ARFGEF2* gene was described in two consanguineous pedigrees in which affected children had microcephaly, severe delay, and early-onset seizures [12]. PNH has been described in association with known genetic syndromes and a number of copy number variants (CNVs) in patients with variably impaired cognitive skills (Table 133.1).

133.3.1.2 Phenotype

Although most patients with PNH come to medical attention because they have focal epilepsy of variable severity, there is a wide spectrum of clinical presentations, including several syndromes with mental retardation and dysmorphic facial features. There is some correlation between the size of PNH and the likelihood of concomitant structural abnormality of the

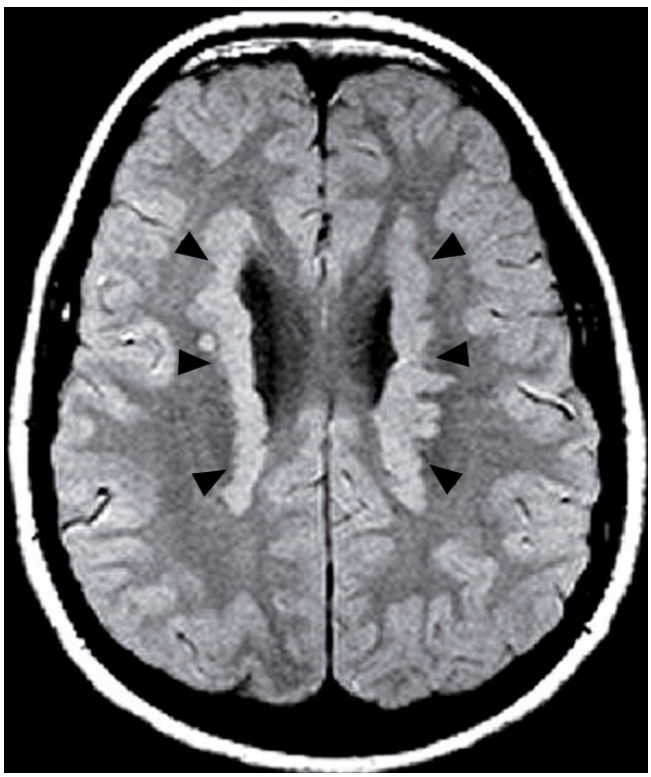

Fig. 133.2 Brain MRI: Axial section. Typical, classical bilateral PNH in a woman with a missense *FLNA* mutation. Bilateral nodules of subependymal heterotopia are contiguous and rather symmetric, extensively lining the ventricular walls (*arrowheads*)

cortex and clinical severity [10] but there seems to be no correlation between the size and number of heterotopic nodules and cognitive outcome. Most female patients with PNH due to *FLNA* mutations have epileptic seizures, with normal or borderline cognitive level. However, patients with even small isolated nodules caused by unknown genetic abnormalities or by copy number variations and severe cognitive impairment have been reported.

133.3.1.3 Laboratory Investigations

FLNA mutation analysis should be performed in patients with "classical" bilateral PNH. When autosomal recessive PNH associated with microcephaly is suspected, *ARFGEF2* mutation analysis should be performed. Patients, who present with PNH associated with other brain malformations or extraneurological defects, should be studied with array-based comparative genomic hybridization (array-CGH), an emerging high-resolution and high-throughput molecular genetic technique that allows genome-wide screening for chromosome alterations (i.e. genomic deletions/duplications).

133.3.1.4 Genetic Counselling

Classical PNH is much more frequent in women and likely to be due to *FLNA* mutations. Among carrier women, about half have *de novo FLNA* mutations, whereas the remaining half have inherited mutations. Although maternal transmission is much more likely, father-to-daughter transmission is possible. Given that germline mosaicism of *FLNA* has never been reported, the recurrence risk (for other children) seems to be very low when a mutation is found in the proband but neither parent is a carrier. Counselling is very difficult when PNH is not related to either *FLNA* or *ARFGEF2*, array-CGH study for the search of copy number variations is advised. The number of known cases of familial PNH unrelated to these genes is extremely low.

133.4 Polymicrogyria Phenotypes and Genetics

The term "polymicrogyria" (PMG) defines an excessive number of abnormally small gyri that produce an irregular cortical surface with lumpy aspect [3]. PMG can be localized to a single gyrus, involve a portion of one hemisphere, be bilateral and asymmetrical, bilateral and symmetrical or diffuse. The imaging appearance of polymicrogyria varies with the patient's age. In newborns and young infants, the malformed cortex is very thin with multiple, very small undulations. After myelination, PMG appears as thickened cortex with irregular cortex-white matter junction [2]. Polymicrogyria is associated with a wide number of patterns and syndromes and with mutations in several genes (Table 133.1). Various PMG syndromes have been described, which have been designated according to their lobar topography [2].

Bilateral perisylvian polymicrogyria (BPP) (Fig. 133.3a, b) is the most frequent form. It is associated with mild to moderate mental retardation, epilepsy, and impaired oromotor skills. Most cases are sporadic but genetic heterogeneity is suggested [2, 13]. BPP, frequently asymmetric and with a striking predisposition for the right hemisphere, has also been reported in children with 22q11.2 deletion [2].

Bilateral frontoparietal polymicrogyria (BFPP) (Fig. 133.3c) has been reported in families with recessive pedigrees and has been associated with mutations of the *GPR56* gene

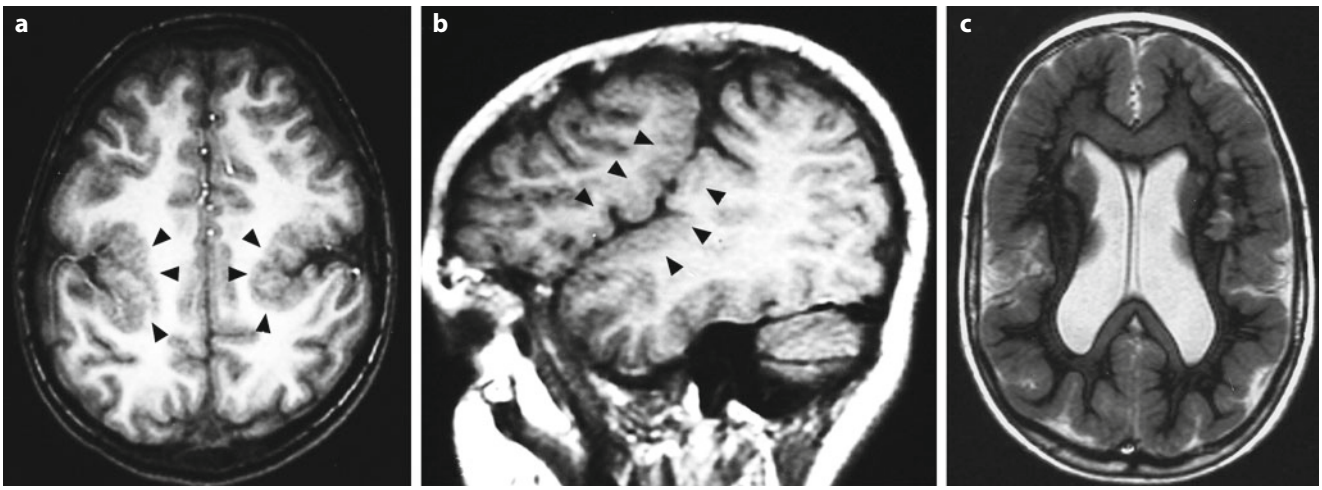

Fig. 133.3 Brain MRI scan in two patients with PMG (**a** and **b** belong to the same patient). **a** Axial section, BPP. The sylvian fissures are open and the perisylvian cortex is thickened and irregular (*arrowheads*). **b** Sagittal section. Note the abnormal vertical orientation of the sylvian fissure, which appears to be fused with the rolandic fissure. **c** Axial section. BFPP in a girl with a *GPR56* mutation and Lennox-Gastaut syndrome

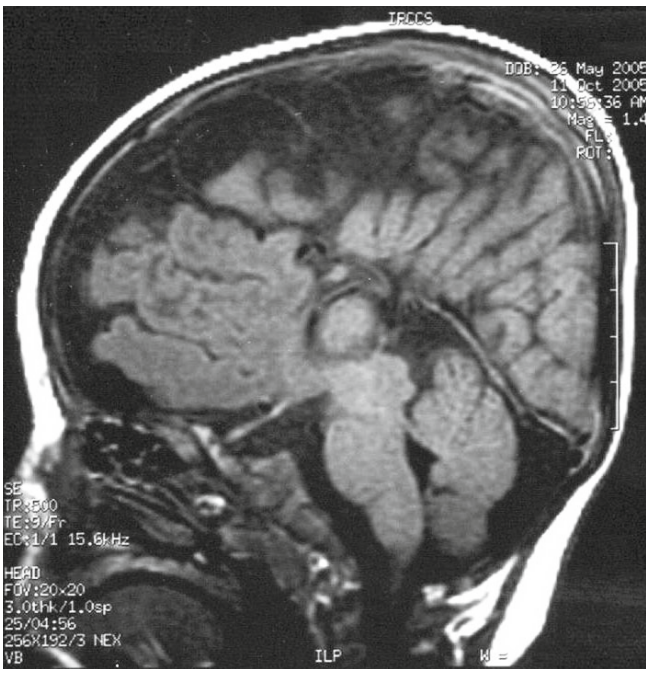

Fig. 133.4 T1 weighted sagittal MRI scan of the brain of a 5 months old girl with Aicardi syndrome and intractable infantile spasms Note the extremely hypoplastic corpus callosum with an extensive area of polymicrogyria involving the frontal lobe. There is a posterior fossa cyst

[14]. The imaging characteristics of BFPP resemble those of the cobblestone malformative spectrum (muscle-eye-brain disease and Fukuyama congenital muscular dystrophy) [2].

Mutations of the *TUBB2B* gene have been associated with asymmetric polymicrogyria [15] but genotype-phenotype correlations need to be clarified. Recently, different types of PMG as part of complex syndromes have been associated with pathogenic CNVs (Table 133.1) [16].

Aicardi syndrome is seen exclusively in females, with the exception of two reported males with two X-chromosomes. It is thought to be caused by an X-linked gene with lethality in the hemizygous male. However, the genetic basis is still unknown. Clinical features include severe mental retardation, infantile spasms and chorioretinal lacunae. Neuropathological findings are consistent with a neuronal migration disorder and include diffuse unlayered polymicrogyria with fused molecular layers, agenesis of the corpus callosum and nodular heterotopias in the periventricular or subcortical region. Microgyri are packed and usually not visible at MRI (Fig. 133.4).

Regrettably, while several causal genes for polymicrogyria have now been discovered, they only account for a small minority of cases and clinical testing is available for only a few. Genetic analysis and counselling in polymicrogyria is still problematic, considering anatomic and etiologic heterogeneity.

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134

Congenital Malformations of the Brain: Prenatal Diagnosis, Spectrum and Causes

Elie Saliba and Christian Paillet

Defective nervous system development results in a diverse group of diseases, ranging from major malformations that are incompatible with postnatal life to moderate or severe disabilities (Table 134.1). The following pages review the main categories of central nervous system (CNS) malformations. A detailed description of the clinical and neuropathological aspects of these defects is beyond the scope of the present chapter.

Table 134.1 Cerebral development and major disorders

Developmental stage	Disorders
Primary neurulation (3–4 weeks' gestation)	Neural tube defects
Prosencephalic development (23 months' gestation)	Holoprosencephaly Agenesis of the corpus callosum
Neuronal proliferation (34 months' gestation)	Microcephaly
Neuronal migration (35 months' gestation)	Lissencephaly Polymicrogyria Schizencephaly Other migration disorders
Myelination (mainly after birth)	White matter hypoplasia

134.1 Prenatal Diagnosis

Suspicion of a congenital malformation may arise on clinical grounds or because of an abnormal result from a routine prenatal investigation. A pregnancy may be at high risk of abnormality because of a particular family history, the advanced age of the mother or some acquired etiologies (Table 134.2). Higher-risk groups for chromosome abnormalities include older mothers, those with a previous chromosomally abnormal child, and when one parent is a translocation carrier. Usually, these women are offered chorion villus sampling or amniocentesis. Ultrasonography is the method of choice for prenatal scanning of fetal anomalies. High-frequency ultrasonography allows visualization of the normal and abnormal development of the embryo or fetus. However, there remain circumstances in which ultrasound data obtained are limited or technically difficult, for example in oligohydramnios and unfavorable position of the fetus. Moreover, some subtle parenchymal abnormalities cannot be seen on ultrasound. Magnetic resonance imaging (MRI) may be a useful adjuvant when ultrasound is indeterminate. MRI is especially useful in cases in which fetal ventriculomegaly is associated with other CNS malformations. Other anomalies such as

Table 134.2 Example of acquired etiologies of human nervous malformations; neural tube defects (NTD); intrauterine growth restriction (IUGR)

Agent	Most common congenital anomalies
Drugs	
– Alcohol	Neuronal migration disorders; Fetal alcohol syndrome; IUGR; facial anomalies
– Cocaine	Microcephaly; IUGR
– Isotretinoin (Accutane)	NTD; craniofacial malformations
– Methotrexate	Hydrocephalus; myelomeningocele; skeletal anomalies
– Phenytoin (Dilantin)	Microcephaly; cleft lip/palate; facial anomalies
– Valproic acid	NTD; facial anomalies
Infectious agents	
– Cytomegalovirus	Microcephaly; hydrocephalus; brain calcifications
– Herpes virus type 1 and 2	Hydranencephaly
– Human immune-deficiency virus	Microcephaly; IUGR
– Rubella virus	Microcephaly; IUGR; heart abnormalities
– *Toxoplasmosa gondi*	Microcephaly; ventriculomegaly; brain calcifications
– Varicella virus	Hydrocephalus

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

lissencephaly and schizencephaly, and also more subtle parenchymal migration disorders such as heterotopia and polymicrogyria have been visualized with MRI.

134.2 Holoprosencephaly: Spectrum and Causes

Subdivision of the early embryonic forebrain to form the bilateral telencephalic vesicles, the precursors of the cerebral hemispheres, is defective in holoprosencephaly. At its most severe, the forebrain is completely undivided (alobar holoprosencephaly) leading to a monoventricle and non-separation of the thalami. Non-separation of these structures declines in less severe forms (e.g., semi-lobar holoprosencephaly). Holoprosencephaly is usually associated with craniofacial malformations such as brachycephaly, microcephaly and abnormal facial development (Table 134.3). In the most severe form, holoprosencephaly may be accompanied by failure of separation of the optic vesicles and eye primordia, yielding the birth defect cyclopia. In less severe forms, the most noticeable abnormalities are often a reduction of midline craniofacial features, with close-set eyes (hypotelorism) or a single central incisor tooth. The detection rate by routine fetal anomaly scan is high for both the lobar and semilobar forms of holoprosencephaly, even in the first trimester [1]. Several chromosomal regions have been linked to holoprosencephaly. The genetic basis of most of the chromosomal linkages in holoprosencephaly has now been elucidated. Several of the causative genes are transcription factors, (*ZIC2*, *SIX3* and *TGIF*) [2]. These genes are known to be expressed in the embryonic brain.

134.3 Neural Tube Defects

Failure of neural tube closure results in malformations termed neural tube defects (NTD). In craniorachischisis, the most severe type of NTD, the neural tube fails to close along most of the body axis, although the forebrain usually closes normally. If the neural tube fails to close specifically in future brain, exencephaly results, which is concerted to anencephaly owing to neurodegeneration in later gestation. In contrast, if the low spine is primarily affected, this leads to open spina bifida. Related to these open CNS defects are a series of closed defects in which the neural tube and/or meninges herniate through an opening in the skull or in the neural arches of the vertebral column. Brain herniation yields a defect called encephalocele, while herniation of the spinal cord is termed meningocele. A further category of neural tube defect are so-called occult spina bifida, which mainly affect the low spinal region and are skin-covered lesions in which the spinal cord may be split or tethered to the surrounding tissues, often in association with a bony spur or a fatty mass (lipoma). While covered lesions are protected from the potentially toxic amniotic environment, open neural tube defect lesions undergo erosion of the exposed neuroepithelium so that, by the late stages of gestation, the region of affected nervous system is largely degenerate, leading to severe disability or death after birth. Surgery of the human fetus during pregnancy, with the aim of covering the neural tube defect lesion with muscle and skin, has shown that this process of degeneration can be halted [3], minimizing damage to the exposed CNS but not recovering function.

Neural tube defects (NTD) are not only very heterogeneous in morphology but also in etiology [4]. Causes include chromosome abnormalities, single mutant genes, teratogens, maternal predisposing factors and multifactorial inheritance. The high recurrence in siblings and in close relatives of individual with NTD suggests a strong genetic basis, although there is a marked lack of large families with NTD, arguing against causation based on single genes. It has been suggested, therefore, that NTD have a multifactorial causation, with many variants interacting to determine individual risk of NTD, and with a marked contribution of environmental factors, both exacerbating and preventive. Despite the many genetic loci that have been implicated in mouse NTD, few human genes have so far been definitively shown to predispose to human NTD. The best known of these is the gene encoding 5, 10-methylene tetrahydrofolate reductase (MTHFR), an enzyme of folic acid metabolism. A polymorphic, variant of the MTHFR gene (the C677T variant) exhibits a higher frequency among NTD cases and their families than among normal controls in several populations and seems responsible for imparting an increased risk of NTDs, especially in combination with a low folate and/or vitamin B12 level during pregnancy [5].

Many environmental factors have been suggested to play a role in NTD etiology. [4]. Nutritional deficiency, maternal diabetes and the use of certain therapeutic drugs such as anticonvulsants and insulin are known risk factors. Periconceptional supplementation of folic acid lowers the occurrence and recurrence risks of human NTD. The mechanisms involved in NTD due to a lower maternal folate status may be defective folic acid mechanism in the mother [7]. A list of recognized cause of NTD in human is presented Table 134.4.

Table 134.3 Facial defects in holoprosencephaly

Cyclopia	Single eye or single orbit Arrhinia with proboscis
Ethmocephaly	Extreme hypotelorism Arrhinia with proboscis
Cebocephaly	Orbital hypotelorism Proboscis-like nose, no cleft
Median cleft	Orbital hypotelorism Flat nose
Agnathia-astomia	Hypoplasia or absence of the mandible Small or absent mouth Abnormal position of the ears

Table 134.4 Examples of recognized cause of NTD in human

Causes	Examples
Chromosome abnormalities	Trisomy 13 Trisomy 18 Various unbalanced chromosome rearrangements, ring chromosomes, triploidy
Single mutant genes: autosomal recessive	Walker-Warburg syndrome Jarco-Levin syndrome Meckel-Gruber syndrome Robert syndrome
Teratogens	Valproic acid Carbamazapine (possible) Hyperthermia (possible)
Maternal predisposing factors	Diabetes mellitus: anencephaly more frequent than spina bifida

134.3.1 Prenatal Diagnosis

NTD can be detected by ultrasound and by analysing the α-fetoprotein level in amniotic fluid or maternal serum. Increased levels of α-fetoprotein are also found in other fetal anomalies such as gastroschisis, esophageal and intestinal atresia, in sacrococcygeal teratoma and in Turner syndrome, but decreased levels are found in trisomies 21 and 18. Moreover, the α-fetoprotein method has a high false-positive rate and a relatively poor sensitivity. Ultrasound is the main method for detection of NTD. Prenatal diagnosis of a myelomeningocele is usually prompted by the recognition of the associated Chiari II malformation. The most efficient sonographic findings, indicating a Chiari II malformation, are the diagnostic "lemon" and "banana" signs (Fig. 134.1) [8]. The lemon sign of the head has become the diagnostic key for detecting spina bifida in the second trimester. The banana sign represents the cerebellum surrounding stem in a small posterior fossa. The lemon sign is transient and usually not present anymore by the end of the second trimester. It represents an abnormal cranial vault that is narrowed rostrally, and results from low pressure in the ventricular cavity due to loss of CSF through the open NTD to the amniotic cavity. With the introduction of transvaginal ultrasound, detailed diagnosis became possible in the early fetal period and even around the end of the embryonic period [9].

134.4 Developmental Disorders of the Cerebellum

Anatomically, cerebellar malformations may be classified into unilateral and bilateral abnormalities. Unilateral cerebellar malformations are most likely due to acquired insults, such as intracerebellar bleeding associated with prematurity. Depending on the part of the cerebellum involved, bilateral cerebellar malformations may be further classified into midline or vermis malformations, and malformations affecting both the vermis and the cerebellar hemispheres [10]. The combination of pontine hypoplasia with cerebellar malformation is considered as a separate group, i.e., the pontocerebellar hypoplasia.

134.4.1 Midline or Vermis Malformations

Agenesis or hypoplasia of the vermis may be found in a large number of malformations of the brain [11], including the Dandy-Walker malformation and syndromes with agenesis of

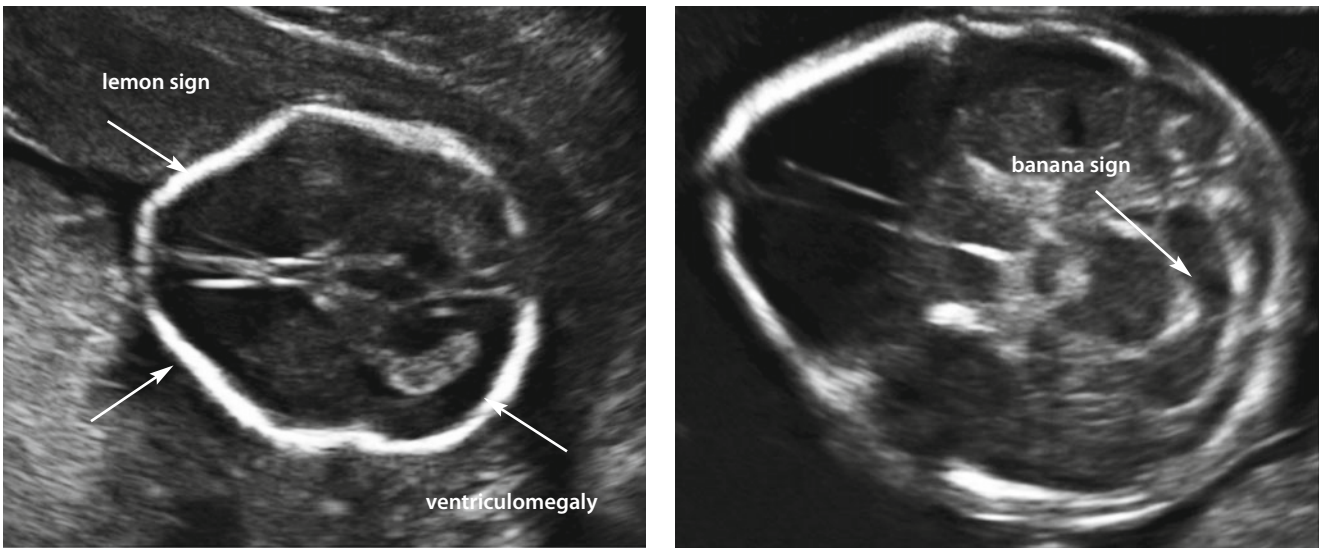

Fig. 134.1 The cranial signs ("lemon" and "banana") associated with open spina bifida

the vermis as a constant feature, such as the Joubert syndrome and Walker-Warburg syndrome, and a large group of syndromes in which absence of the vermis may occur, such as Meckel-Gruber and Smith-Lemli-Opitz syndromes.

The Dandy-Walker malformation is characterized by the following triad: cystic dilatation of the fourth ventricle and an enlarged posterior fossa; varying degrees of vermian aplasia or hypoplasia; and hydrocephalus (Fig. 134.2). Hydrocephalus, associated with a bulging occiput, is unusual at birth but is present by 3 months of age in about 75% of patients [12, 13]. Mental retardation and seizures have been reported in up to 50% of cases with a Dandy-Walker malformation [14]. Associated CNS malformations are present in up to 68% of the cases, the most common of which is agenesis or hypogenesis of the corpus callosum. Other CNS malformations include neuronal heterotopias, polymicrogyria, schizencephaly, occipital encephaloceles and lumbosacral meningoceles [12].

Most cases of Dandy-Walker malformation are sporadic. The etiology remains unknown. Distinguishing inheritable syndromes from isolated cases of vermian-cerebellar hypoplasia is important for genetic counseling. Bordarier and Aicardi classified the genetically heritable syndromes with complex vermian-cerebellar hypoplasia into two groups: 1) those in which vermis aplasia is a constant feature, the most common entities being Joubert syndrome, Walker-Warburg syndrome and related cerebro-oculomuscular syndromes; and 2) those in which vermis aplasia is an occasional component, such as Meckel-Gruber syndrome, Coffin-Siris syndrome, Smith-Lemli-Opitz syndrome and Ellis-van Crevel syndrome, all autosomal recessive traits [12]. Dandy-Walker syndrome has recently been shown to be related in some cases to genetic disorders of two members of the *ZIC* gene family, which encode zinc finger transcription factors [15].

Joubert syndrome is a relatively rare, autosomal recessive disorder defined by vermis hypoplasia, hypotonia, developmental delay and at least one of two additional manifestations: abnormal breathing pattern (hyperpnea intermixed with central apnea in the neonatal period) or abnormal eye movements [16]. In Joubert syndrome, ataxia, mental retardation and behavioral disorders become manifest in late infancy and childhood [11]. The prognosis of Joubert syndrome is poor; a causative gene was recently identified [17].

134.4.2 Cerebellar Hypoplasia

In cerebellar hypoplasia, the cerebellum does not reach its normal size. Global cerebellar hypoplasia may result from a variety of exogenous or endogenous factors. It is found as a result of intrauterine exposure to drugs (e.g., phenytoin) or irradiation, and as an autosomal recessive trait in a variety of chromosomal disorders such as trisomies 13, 18 and 21 [19]. Cerebellar hypoplasia may also be found in various complex malformations involving the brain and other systems. Primary degeneration of the granular layer of the cerebellum or granular layer aplasia (GLA) occurs as an autosomal regressive disorder [20]. Patients with features consistent with GLA have been found to have elevated levels of serum asialotransferrin and heterozygous deficiency of phosphomannomutase type 2 (PMM2). This deficit is involved in the congenital disorders of glycosylation (CDGs) [21].

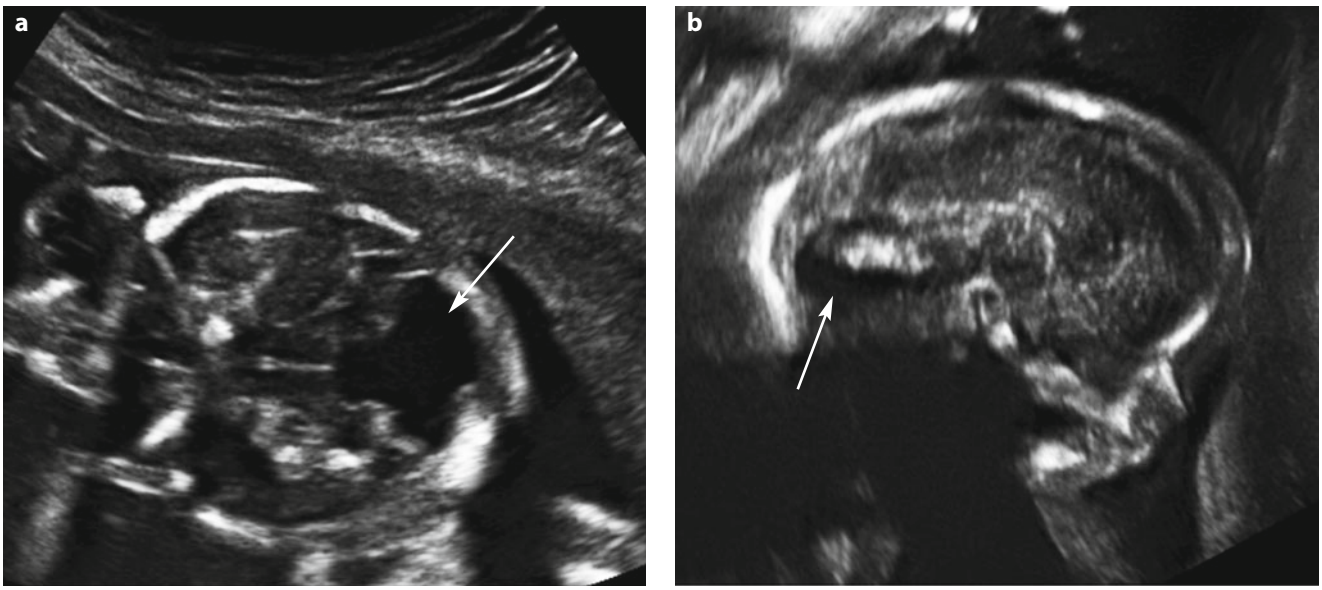

Fig. 134.2 Dandy-Walker malformation: **a** axial plane demonstrating a large cisterna communicating with the area of the fourth ventricle (*white arrow*); **b** sagittal view demonstratin a large cisterna magna

134.4.3 Pontocerebellar Hypoplasia

The pontocerebellar hypoplasias form a large group of disorders characterized by a smaller volume of the pons and varying degrees of cerebellar hypoplasia up to near-total absence of the cerebellum. Most types of pontocerebellar hypoplasia arise in the fetal period, suggesting a rhombic lip defect and the *MATH1* gene as candidate for this disorder. Most pontocerebellar hypoplasias are autosomal recessive disorders [22].

134.5 Neuronal Migration Disorders

Neuronal migration consists of nerve cells moving from their original site in the ventricular and subventricular zones to their final location. Regulation of timing and direction of these simultaneous migrations is carried out with extreme precision. Neurons originating in the cortical ventricular zone migrate radially to form the cortical plate and mainly become projection neurons [23]. Migration of neocortical neurons occurs mostly between the fifth gestational week, when the telencephalic vesicle appears, and 22 weeks of gestation in the human [24]. The neurons that migrate first will stop in the deepest cortical layers, those which migrate afterwards pass through the layers formed previously to form the outer cortical layers, according to a migration scheme defined as "inside-out". Neocortical migration neurons can adopt different types of trajectories: a large proportion of neurons migrate radially along radial glial guides, from the germinative zone to the cortical plate. Another important group of neurons adopt a tangential trajectory at the level of the ventricular or subventricular germinative zones before adopting a classic radial migrating pathway along glial guides. Tangentially migrating neurons have also been located at intermediate zone level (prospective white matter). Three large classes of genes underlie the vast majority of neuronal migration disorders that are seen clinically: 1) those involving the formation of the extracellular environment encountered by migrating neurons and axons; 2) those encoding for intracellular signaling mechanisms; and 3) those encoding the intracellular system that mediates cellular and axonal physical movements [25]. Another group of genes is involved in encoding for the enzymatic regulator of glycosylation, which, in turn, seems to provide stop signs for migrating neurons [26]. A list of genes implicated in neuronal migration disorders is presented in Table 134.5.

The neuronal migration disorders are a heterogeneous group of congenital brain defects. They are perhaps the most common form of CNS malformations. They include lissencephaly, heterotopia, focal cortical dysgenesis, polymicrogyria and schizencephaly.

134.5.1 Lissencephaly

Lissencephaly (LIS), which literally means smooth brain, consists of a set of rare brain disorders characterized by the lack of normal cortical convolutions. The severity ranges from absence (agyria) to reduction (pachygyria) of normal gyral pattern. On the basis of etiologies and associated malformations, five groups of lissencephaly can be identified: classical lissencephaly, cobblestone lissencephaly, X linked lissencephaly with agenesis of the corpus callosum, lissencephaly with cerebellar hypoplasia, microlissencephaly [27]. The onset of lissencephaly is considered to occur no later than the 12th–16th week of gestation [28].

The various clinical subtypes of lissencephaly have different risks of recurrence in siblings. Isolated lissencephaly is causally heterogeneous. The recurrence risk is 5–7% [28]. The recurrence for Miller-Dieker syndrome depends on results of chromosome and DNA analysis; it is not inherited as an autosomal-recessive trait. The recurrence risk is 25% for isolated lissencephaly with neonatal death, Norman-Roberts syndrome, Fukuyama congenital muscular dystrophy and Walker-Warburg syndrome. The recurrence risk for unusual lissencephaly such as lissencephaly with cerebellar hypoplasia

Table 134.5 Genes implicated in neuronal migration disorders

Neuronal migration disorder	Gene	Gene function	Location
Lissencephaly (Miller-Dieker and isolated lissencephaly sequence)	*LIS1*	Microtubule activating protein	17p13.3
X-linked lissencephaly	*DCX* (double-cortin)	Microtubule stabilizing protein	Xq22.3-q23
X-linked lissencephaly with ambiguous genitalia	*ARX*	Homeobox transcription factor	Xp22.13
Autosomal recessive lissencephaly	*RELN* (Reelin)	Extracellular matrix signaling protein	7q22
Cobblestone (Type 2) lissencephaly (Fukuyama muscular dystrophy)	*FCMD* (fukutin)		
Cobblestone (Type 2) lissencephaly (Walker-Warburg syndrome)	*POMT1*	α-Mannosyl transferase enzyme	9q34.1
Nodular periventricular heterotopia	*FLNA* (Filamin –A)	Actin binding protein	Xq28

or microcephaly may be as high as 25%. The recurrence risk is 50% for brothers of males having X-linked lissencephaly with microcephaly. Genetic counseling is always indicated for families with children with lissencephaly [28].

134.5.2 Neuronal Heterotopia

Neuronal heterotopia is a neuronal migration disorder characterized by a cluster of disorganized neurons in abnormal locations and it includes three main groups: periventricular nodular heterotopia (PNH), subcortical heterotopia (SBH) and marginal glioneural heterotopia. A classification of neuronal heterotopia is presented Table 134.6 [29].

Periventricular nodular heterotopia (PNH) is a rare malformation in which group of neurons are abnormally positioned close to the ventricular cavities. PNH can be caused by genetic mutations or extrinsic factors such as infections or prenatal injuries [30]. X-linked periventricular heterotopia results from mutation in the gene encoding Filamin-A (FLNA), an actin-cross-linking phosphoprotein that interacts with Filamin-B and Filamin-A-interacting protein (FILIP) to regulate the actin reorganization necessary for neuronal migration [31]. When the function of this protein complex is disturbed, neuroblasts are unable to initiate migration. Mutation of the FLNA gene (Xq28) causes bilateral PNH (OMIM # 300049) in the majority of patients; this form is often fatal for males, therefore explaining the female preponderance. The autosomal recessive form of PNH (OMIM # 608097) is caused by mutation in the *ARFGEF2* gene localized at 20q13.13, which encodes for the protein brefeldin-inhibited GEF2 (BIG2).

PNH has also been associated with chromosomopathies such as duplication of chromosome 5 (OMIM # 608098) and deletion on chromosome 6 or 7 [32].

MRI in patients with X-linked dominant mutation shows bilateral symmetric nodules adjacent to the lateral ventricular walls. Unilateral PNH is commonly located in the posterior paratrigonal zone of the lateral ventricles.

Table 134.6 Classification of heterotopia from Barkovich [29]

1. *Periventricular heterotopia*
   a. Periventricular nodular heterotopia (PNH)
      i. Bilateral PNH with FLN1 mutations
      ii. PNH with mutations of chromosome 5
      iii. PNH with bilateral polymicrogyria-perisylvian and posterior subtypes
   b. Periventricular linear heterotopia (unilateral or bilateral)
2. *Subcortical heterotopia*
   a. Large subcortical heterotopia with cortical infolding, abnormal cortex, hypogenetic corpus callosum
   b. Pure subcortical heterotopia nodules
   c. Columnar heterotopia
   d. Excessive single neurons in white matter
3. *Marginal glioneuronal heterotopia*

SBH is caused by alterations in two genes: *LIS1* at 17p13.32 (32) and *DCX* at Xq22.3-q23.3. MRI of the brain in SBH demonstrates two parallel layers of gray matter, a thin outer ribbon and a thick inner band, separated by a very thin layer of white matter [30].

134.5.3 Polymicrogyria

Polymicrogyria (PMG) is a cortical malformation characterized by an irregular brain surface with an excessive number of small and partly fused gyri separated by shallow sulci.

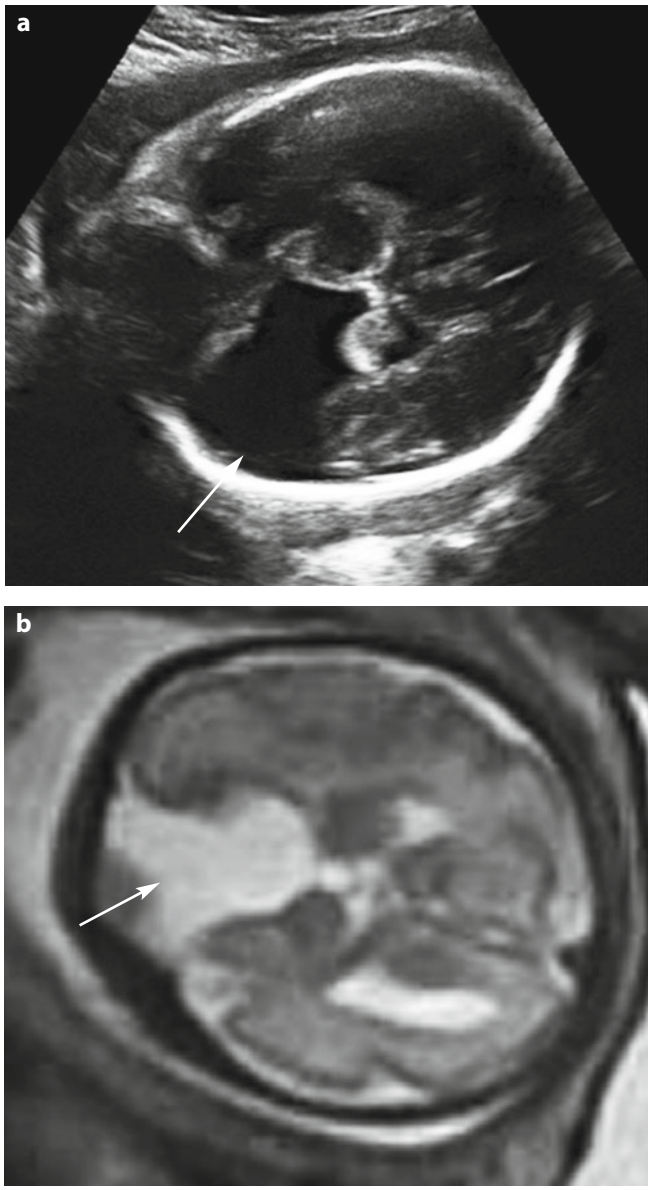

Fig. 134.3 Schizencephaly: **a** axial view (*arrow*); **b** magnetic resonance at 32 weeks (*arrow*)

PMG can be focal or diffused, unilateral or bilateral. It can be an isolated lesion or associated with other brain malformations such as heterotopia, white matter lesions or a part of several multiple congenital mental retardation syndromes [34]. Two types of polymicrogyria can be identified by histology: a simplified four layered form and an unlayered form [35]. PMG was found to be associated with congenital CMV infection, perinatal hypoxia-ischemia, twin-twin transfusion, loss of twin in utero or maternal drug ingestion [36]. PMG was found in association with genetic disorders such as Aicardi syndrome, Zellweger and Walker-Warburg syndromes or chromosomal abnormalities such as 22q11 deletion and trisomy 13.

134.5.4 Schizencephaly

Schizencephaly (SZC; OMIM # 269160) is a structural abnormality of the brain, characterized by congenital clefts spanning the cerebral hemisphere from the pial surface to the lateral ventricle and lined by cortical gray matter (Fig. 134.3) [37]. Cleft localization varies widely but perisylvian region is more often involved [38]. SCZ can be unilateral or bilateral. The etiology of this malformation is not well established and several causes including genetic, vascular, toxic, metabolic and infectious factors might be involved. Exposure in utero to toxins and CMV infections has been incriminated [37, 39, 40].

134.6 Disorders of CNS Cell Number: Microcephaly

Human microcephaly comprises a heterogeneous group of conditions that are characterized by a failure of normal brain growth. Microcephaly is usually defined as a condition in which the size of the head, measured by the occipitofrontal circumference (OFC), is greater than two standard deviations below the mean for gestational age and gender. The etiology of microcephaly can be broadly divided into environmental and genetic cause [41]. Common environmental causes are congenital infections such as cvytomegalovirus (CMV), intrauterine exposure to teratogenic agents, and hypoxic-ischemic injury in the fetal period (Fig. 134.4). The genetic causes of congenital microcephaly can be further subdivided, based on the presence of normal versus abnormal brain architecture as found on MRI examination, and its combination with non-CNS abnormalities. Microcephaly is a characteristic feature in holoprosencephaly and lissencephaly. Decreased neuronal proliferation may lead to microcephaly with a normal to thin cortex (primary microcephaly or microcephaly vera, and extreme forms of microcephaly with a simplified gyral pattern), microlissencephaly (extreme microcephaly with thick cortex) and microcephaly with polymicrogyria or other cortical dysplasias.

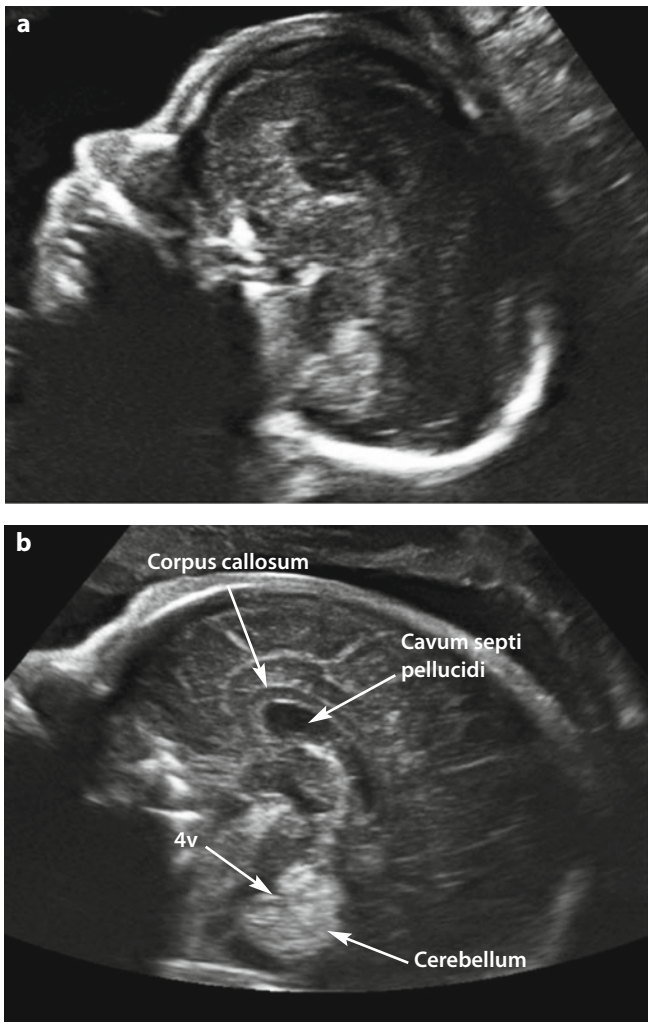

Fig. 134.4 Sagittal planes at 32 weeks: **a** complete agenesis of the corpus callosum; **b** normal sagittal plane

Investigation of patients with microcephaly includes evaluation for prenatal exposure to teratogens, especially alcohol, drugs and isotretinoin (a vitamin A analog), and assessment of the family history, birth history and associated dysmorphic conditions. Laboratory studies should include toxoplasmosis, rubella virus, CMV and herpes simplex; evaluation of maternal metabolic disorders; and chromosome analysis (Table 134.7)

Table 134.7 Assessment of a newborn with microcephaly

Family history	Parental consanguinity, family history, antenatal exposure to teratogens including alcohol
Parents' examination	Parental OFC
Child examination	Dysmorphic features
Special investigations	Chromosome analysis, congenital infection screening, metabolic screening, eye examination, brain MRI

134.7 Disorders of Cortical Connectivity: Malformations of the Corpus Callosum

Malformation of the corpus callosum, the large white matter tract that connects the cerebral hemispheres, is a common congenital anomaly [42]. If normal development of the corpus callosum is disturbed, it may be completely absent (agenetic), partially formed (hypogenetic), formed in a defective way (dysgenetic) or contain too few axons (hypoplastic).

Agenesis of the corpus callosum (ACC) occurs in about one to three per 1000 births, is usually sporadic, and may be transmitted as a sex-linked, autosomal-dominant, or autosomal-recessive trait [43]. Prenatal diagnosis of ACC can be established by 20 weeks' gestation with ultrasonography (Fig. 134.5). In about half of the reported fetal US studies, ACC is an isolated finding; in the remaining studies, other abnormalities or findings suggestive of specific syndromes are found. Male fetuses are more likely to have isolated agenesis that is considered benign. The etiology of ACC has been associated with numerous syndromes (Aicardi syndrome) and several inborn error of metabolism, including non-ketonic hyperglycinemia and fetal alcohol syndrome. ACC may be observed in various malformations of cortical development, including the lissencephalies, polymicrogyria and schizencephaly. Chromosomal disorders, particularly trisomies 8, 13 and 18 are also associated with ACC [44, 45]. Clinically, the extent and nature of neurologic compromise result from the congenital absence of the corpus callosum and the associated brain abnormalities.

134.8 Intracranial Arachnoid Cysts

Intracranial arachnoid cysts are benign, non-genetic developmental cysts that contain spinal fluid and occur within the arachnoid membrane [46]. The walls of the cyst result from splitting of the arachnoid membrane. There is no epithelium lining the cystic place and the fluid it contains is translucent. The cysts occur in proximity to arachnoid cisterns, most often in the sylvian fissure. Arachnoid cysts occur most often in males and in patients with Marfan syndrome and there are few reports of familial middle and posterior fossa arachnoid cysts. The mechanism of formation during embryogenesis is uncertain. Macrocephaly is the major presenting symptom and associated features include the following: cranial asymmetry, aqueductal stenosis and agenesis of the corpus callosum [46].

134.9 Hydrocephalus and Ventriculomegaly

Hydrocephalus signifies dilated lateral ventricles resulting from an increased amount of CSF inside the ventricles and increased intracranial pressure, while ventriculomegaly is a dilatation of lateral ventricles without increased intracranial

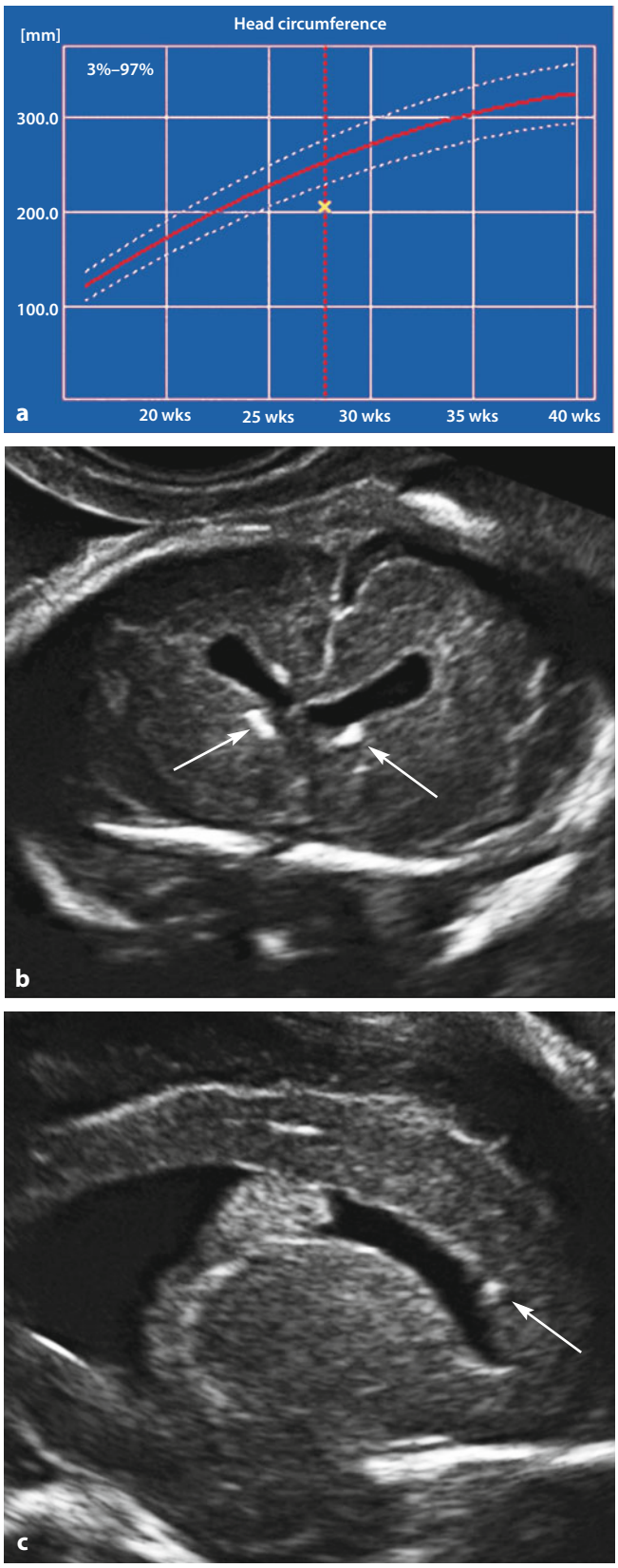

Fig. 134.5 Microcephaly in a 28 weeks' fetus with CMV infection. **a** head circumference: 203 mm (-2SD); **b** coronal view showing mild ventriculomegaly and calcifications (*white arrows*); **c** parasagittal view showing calcification

pressure, due to cerebral hypoplasia or CNS anomaly such as agenesis of the corpus callosum [47]. Ventriculomegaly is defined as an enlargement of the lateral cerebral ventricles. It can be regarded as a non-specific marker of abnormal brain development and is encountered with many different cerebral anomalies. Evaluation of the integrity of the cerebral lateral ventricles is therefore of particular importance while screening for fetal cerebral anomalies. Although many different approaches to the evaluation of the integrity of lateral ventricles have been proposed, measurement of the internal width of the atrium of the lateral ventricle at the level of the glomus of the choroid plexus is currently favored [48–50]. Under normal conditions, the measurement is less than 10 mm, while a value of more than 15 mm indicates severe ventriculomegaly, which is almost always associated with an intracranial malformation at birth. The outcome of these fetuses is variable and depends largely upon the underlying etiology of the ventricular dilatation. The available studies suggest that fetuses with isolated severe ventriculomegaly have an increased risk of perinatal death and a probability of severe neurologic sequelae in the range of 50% of survivors [51]. An intermediate value for the atrial width, 10–15 mm, is commonly referred as to as mild ventriculomegaly and is associated with a much increased probability of cerebral and extracerebral malformations, aneuploidies and infections and therefore should be carefully evaluated in an expert center. Fetuses with isolated mild ventriculomegaly usually have a good outcome and, in most infants, the ventricles return to normal size during pregnancy. However, these infants also run an increased risk of neurologic compromise and, in some cases, develop severe cerebral anomalies in the last of the gestation or after birth, including hydrocephalus, white matter injury and cortical plate abnormalities [52]. The risk is particularly increased when the atria width is greater than 12 mm, when the dilatation affects both ventricles and in females [53]. It has been suggested that the term mild ventriculomegaly should be limited only to cases with atrial measurements of 10–12 mm, while values of 12.1–15 mm should be referred to as moderate ventriculomegaly, as they tend to have, in general, a worse outcome [54–56].

Congenital hydrocephalus is classified into three categories by causes that disturb the CSF circulation pathway: simple hydrocephalus, dysgenetic hydrocephalus and secondary hydrocephalus.

- Simple hydrocephalus caused by developmental abnormality which is localized within the CSF circulation pathway includes aqueductal stenosis, atresia of foramen Monro and maldevelopment of arachnoid granulation.
- Dysgenetic hydrocephalus indicates hydrocephalus as a result of cerebral development disorder and includes hydranencephaly, holoprosencephaly, porencephaly, schizencephaly, Dandy-Walker malformation, dysraphism, and Chiari malformation.
- Secondary hydrocephalus is caused by an intracranial pathology such as: brain tumor, intracranial infection and intracranial hemorrhage.

Genetic hydrocephalus is rare but it is important in counseling couples on subsequent pregnancies. It is now well recognized that a small proportion of all cases of hydrocephalus show X-linked inheritance. Most of these X-linked cases are known to be caused by mutations in the *L1CAM* gene which is located at Xq28 [55]. The *L1CAM* gene encodes a neuronal cell adhesion molecule, known as L1, which is involved in neuronal migration and axonal extension. Mutation in *L1CAM* can result in several different phenotypes such as X-linked hydrocephalus, hydrocephalus due to stenosis of the aqueduct of Sylvius, the MASA syndrome (mental retardation-aphasia, shuffling gait, adducted thumbs), X-linked complicated spastic paresis and X-linked corpus callosum agenesis.

134.10 Conclusions

Congenital brain malformations are a significant cause of morbidity and mortality. In recent years, significant advances in basic neuroscience research have improved our understanding of the molecular and genetic underpinnings of brain development. Continued advances in genomics research will move us toward an even better understanding of these developmental, processes, with the hope of one day being able to provide parents and clinicians with the information they so desperately need to make informed decisions.

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Biochemical Basis of Hypoxic-Ischemic Encephalopathy

Maria Delivoria-Papadopoulos and Endla Anday

135.1 Introduction

Perinatal hypoxia ischemia is the most common cause of neurologic disease during the neonatal period. Hypoxic ischemic encephalopathy (HIE), is associated with a high mortality and morbidity rate, including cerebral palsy, mental retardation, and seizures [1]. The incidence of perinatal asphyxia is about 1.0–1.5% in most centers and is usually related to gestational age and birth weight. It occurs in 9.0% of infants less than 36 weeks' gestation and in 0.5% of infants more than 36 weeks' gestation [2, 3]. The etiology of perinatal HIE includes those circumstances that can affect the cerebral blood flow in the fetus and newborn compromising the supply of oxygen to the brain. They may develop antepartum (20%), intrapartum (30%), antepartum and intrapartum (35%), or postpartum (10%) [4].

HIE develops in the setting of perinatal asphyxia, which is a multiorgan system disease [2]. Assessment and management of these complications is an integral part of the treatment of perinatal asphyxia/HIE [2]. The present chapter primarily focuses on cellular and molecular mechanisms of the hypoxic neuronal injury in the newborn brain.

A large amount of information has been collected on the fetal cardiovascular and respiratory response to oxygen limitations, giving rise to a better understanding and management of neonatal deterioration induced by hypoxia. Besides these physiologic studies, cellular and biochemical mechanisms that result in brain cell death are being increasingly explored, particularly in the adult with respect to focal (stroke) and global (cardiac arrest) hypoxia-ischemia [4]. These studies have shown that complex and interrelated biochemical alterations are triggered during hypoxia-ischemia in mature subjects that ultimately result in neuronal death. Studies are in progress to investigate mechanisms of hypoxic brain injury in the fetus and newborn brain [5]. To focus on cellular and molecular mechanisms of hypoxic injury in the developing brain, it is important to recognize the factors that may determine the susceptibility of the developing brain to neonatal and perinatal hypoxia.

The determinants of the susceptibility of the developing brain to hypoxia include the lipid composition of the brain cell membrane, the rate of lipid peroxidation, the presence of antioxidant defenses, the development and modulation of the excitatory neurotransmitter receptors such as the N-methyl-D-aspartate (NMDA) receptor, and the intracellular Ca^{++} influx mechanisms. In addition to the developmental status of these cellular components, the response of these potential mechanisms to hypoxia determines the fate of the hypoxic brain cell in the developing brain in the fetus and the newborn. Elucidating basic cellular mechanisms in response to hypoxia of the developing brain will enable the development of novel strategies for preventing or attenuating the deleterious effects of hypoxia in the human newborn. Several excellent reviews on different aspects of hypoxic/ischemic cell injury in the developing brain have been published recently [6, 7].

135.2 Steps of Posthypoxic Brain Injury

Acute or long-term consequences of hypoxic-ischemic encepholopathy are related either to necrosis or to apoptosis of neuronal cells. Necrosis is characterized by passive cell swelling, rapid energy loss, generalized disruption of internal homeostasis leading to eventual lysis of the nucleus, organelles and plasma cell membranes and the release of intracellular components that induce a local inflammatory response, resulting in edema and injury to the neighboring cells. This inflammatory response results in the expression of the cytokines interleukine-1-beta, (IL-beta) and tumor necrosis factor-alpha (TNF-alpha) stimulating oxygen free radicals release from neutrophils, activating microglical cells.

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Necrosis is only one of the mechanisms of cell death following hypoxia or ischemia to the brain. Programmed cell death, or apoptosis, also appears to contribute to cell death following hypoxia-ischemia, especially cell death that occurs days to weeks following the insult [8, 9]. Apoptosis is an active process that requires the activation of a genetic program and specific endonucleolytic digestion of nuclear DNA. In contrast to necrosis, programmed cell death is characterized by cell shrinkage, coarse chromatin aggregation with extensive nuclear DNA fragmentation, nuclear pyknosis and extrusion of membrane-bound cytoplasmic fragments or apoptotic bodies, but is not associated with the lysis of the plasma membrane [10, 11].

Studies in cell culture models have demonstrated that hypoxia can trigger programmed cell death [12]. Programmed cell death as assessed by the cleavage of genomic DNA has also been shown to occur in the brain following focal [8, 13, 14] and global ischemia [9, 15].

The mechanism by which hypoxia causes DNA fragmentation has been extensively studied but is not well understood.

135.2.1 Energy Breakdown

Under normal conditions the oxidative phosphorylation and the synthesis of high energy phosphates like adenosine 5-phosphate or ATP need adequate oxygen supply. Under anaerobic conditions the metabolic cost of ATP production is critically increased leading to a breakdown of energy balance depleting the brain cells of high energy compounds, necessary for energy-dependent metabolic processes in neurons and glial cells.

135.2.2 Excitotoxic Mechanisms

The fall of ATP levels includes a cell membrane depolarization and a disruption of voltage dependent ion-channels allowing excessive amounts of Ca^{++} to enter the cytosol, initiating the release of glutamate consequently activating N-methyl-D-aspaptate (NMDA) receptors. The increased expression/activation of NMDA receptors further enhances cellular calcium influx. This whole mechanism is further accelerated by the dysfunction of the energy dependent reuptake of glutamate both in neurons and in astrocytes in a vicious circle manner.

135.3 NMDA Receptors

Glutamate is the major excitatory amino acid neurotransmitter that contributes to a number of developmental processes such as synaptogenesis, synaptic plasticity, long-term potentiation (LTP), learning and memory as well as neurodegeneration and hypoxia-induced injury [16, 17]. The physiological and pathological effects of glutamate in the central nervous system (CNS) are mediated through its interaction with specific cell membrane receptors, of which the N-methyl-D-aspartate (NMDA), kainate, and AMPA subtypes are the best characterized [18]. The use of specific antagonists [18–21] of the NMDA receptor support the role of receptor activation in long-term potentiation and hypoxic-ischemic cerebral injury. The NMDA-type glutamate receptor is a predominant mediator of excitotoxicity in the immature as compared to the adult brain due to overexpression of the receptor in the developing immature brain [22]. Within the developmental period however, the extent of NMDA receptor mediated processes such as LTP and hypoxia-induced excitotoxicity may depend on the ontogeny of the NMDA receptor sites and subunits leading to altered function of the ion-channel complex. In addition, the function of the receptor may be modified by intracellular mechanisms such as phosphorylation/dephosphorylation, nitration, and pathways of free radical generation.

135.3.1 Structure and Function of The NMDA Receptor

The activity of the NMDA receptor ion-channel complex is regulated by a number of pharmacologically distinct binding sites. The NMDA receptor possesses a neurotransmitter binding site, or recognition site, which binds glutamate or NMDA; a co-activator site which binds glycine; a channel site that binds MK-801 in its open state; a voltage-dependent Mg^{++} binding site; a polyamine site that binds spermine and spermidine; an ifenprodil site, and an inhibitory divalent cation site that binds Zn^{++} [18]. The activity of the receptor ion-channel can also be modified by redox agents [23, 24]. Ligand binding studies indicate that there are two distinct binding sites or states associated with the glutamate recognition site, one that preferentially binds agonists and one that preferentially binds anatognists [25].

The NMDA receptor is associated with a cation-selective ion-channel that gates Na^+, K^+, and Ca^{++} ions and, in the resting state, when blocked by Mg^{++} in a voltage-dependent manner [26, 27], the blockade of the ion-channel complex by glutamate or NMDA is allowed, and the agonist-dependent Ca^{++} influx occurs. The influx of Ca^{++} ions is thought to initiate biochemical processes responsible for both NMDA receptor-induced plasticity in the developing brain and NMDA receptor-mediated excitotoxic cell death [17, 28, 29].

Each of the regulatory sites of the NMDA receptor ion-channel complex is modified during brain development and may alter the site-dependent influx of NMDA receptor-mediated Ca^{++} and site-specific responses of the receptor during development and during hypoxia.

135.3.2 Mechanism of NMDA Receptor Modification During Hypoxia

Brain tissue hypoxia modifies the NMDA receptor recognition, co-activator, and the ion-channel sites. A decrease in the apparent number of NMDA receptors and an increase in receptor affinity for MK-801 were observed in hypoxic fetal guinea pig and newborn piglet brain [30, 31]. In these same studies, glutamate and glycine-dependent activation of the NMDA receptor was decreased and spermine-dependent and basal state receptor activation were increased during hypoxia. In brains of hypoxic newborn piglets, it was noted that hypoxia modified the recognition, co-activator, and modulatory sites of the NMDA receptor ion-channel complex, probably through NO-mediated nitration [31, 32]. Several lines of evidence support this conclusion.

First, in neurons of the central nervous system, neuronal nitric oxide synthase (nNOS) is colocalized with the NMDA receptor [33, 34], thereby favoring nitration of the receptor. Second, nNOS activity is decreased by phosphorylation and increased by dephosphorylation [35, 36], a condition that may be achieved during hypoxia.

Furthermore, dephosphorylation of the receptor will make tyrosine sites available for nitration by peroxynitrite, which is produced by NO and superoxide radicals, both of which are produced during hypoxia. Peroxynitrite-dependent nitration inhibits phosphorylation of proteins [37], indicating possible steric hindrance between the phospho-and nitro-groups on the same tyrosine residue. Thus, dephosphorylation during hypoxia may facilitate peroxynitrite-mediated nitration on the 3-position of tyrosine. In view of these considerations, a critical role of the nitric oxide synthase (NOS) pathway in NO mediated mechanism of hypoxia-induced modification of the NMDA receptor in newborn brain is strongly suggested.

In neurons of the central nervous system, nNOS is colocalized with NMDA receptors [33, 34]. In additions, neuronal NOS is activated by Ca^{++} influx through the NMDA receptor ion-channel, however, nNOS is not efficiently stimulated by activation of non-NMDA receptors that also induce Ca^{++} influx [38]. In synaptic plasma membranes, the nNOS immunoreactivity is associated with the NMDA receptor [39]. The synaptic localization of nNOS in the brain may be mediated by the postsynaptic density protein, PSD-95. Recently, it was demonstrated that nNOS, PSD-95, and NMDA receptor subunit NR2B from the brain coimmunoprecipitate and that the PSD-95 is sufficient to assemble a tight ternary complex with nNOS and the NR2B subunit of the NMDA receptor [40].

In summary, results of these studies indicate that NO production in the brain is preferentially activated by Ca^{++} influx through the NMDA receptor ion-channel and that there is a specific structural and functional link between the NMDA receptor and nNOS.

135.4 Free Radicals

Free radicals are molecular species with unpaired electrons in the outer orbit with a strong tendency to initiate chain reactions that result membrane peroxidation, protein oxidation, nucleic acid oxidation, and cell damage. Normally, more than 80% of the oxygen consumed by the cell is completely reduced by cytochrome oxidase to water without production of oxygen free radicals. The remaining 10–20% undergoes other oxidation reduction reactions in the cytoplasm and mitochondria that produce a superoxide anion radical.

135.4.1 Free Radical Generation in Cerebral Cortex of Newborn Piglets

The production of free radicals during hypoxia was documented by measuring the signal of spin adducts with electron spin resonance spectroscopy. Newborn piglets of 3–5 days were assigned to either normoxia (PaO_2 – 120 mmHg) or hypoxia (PaO_2 < 20 mmHg) for 1 hour. Cortical samples were obtained by biopsy from anesthetized, ventilated piglets.

The data provided direct evidence of increased free radical generation during hypoxia in the newborn model. On the basis of the characteristics of the spin adduct signal the free radical species present in the hypoxic tissue was identified to be an alkoxyl radical.

These studies demonstrate increased free radical generation during hypoxia in the cerebral cortex of the fetus and the newborn, and intervening with the inhibitors of pathways of free radical generation reduced the hypoxia-induced production of free radical species. Alkoxyl radical appears to be the predominant free radical species identified during hypoxia, indicating that free radical-mediated lipid peroxidation is an ongoing event during cerebral hypoxia, a mechanism of hypoxic neuronal injury.

135.4.2 Mechanisms of Free Radical Generation During Hypoxia

There are a number of potential mechanisms of free radical generation under hypoxic conditions. During hypoxia, the increased accumulation intracellular Ca^{++} due to excessive activation of NMDA [41] and non-NMDA receptors is crucial in hypoxia-induced excitotoxicity. Increased intracellular Ca^{++} can initiate a number of biochemical events that could lead to free radical generation and cell death such as: (1) activation of phospholipase A_2 leading to increased generation of oxygen-free radicals from cyclooxygenase and lipoxygenase pathways; (2) activation of NOS, leading to peroxynitrite formation and generation of free radicals; (3) activation of

proteases, leading to conversion of xanthine dehydrogenase to xanthine oxidase and resulting in increased free radical generation; (4) activation of phospholipase C_1 leading to IP3, formation and resulting in the release of Ca^{++} from intracellular stores; and (5) free radical generation further triggering the release of additional excitatory amino acids neurotransmitters as well as influencing the activation of the NMDA receptor ion-channel activity through the redox site.

In addition to Ca^{++} mediation, there are other potential mechanisms of free radical generation during hypoxia such as: (1) reduction of electron transport chain components including ubiquinone (a component that undergoes autooxidation to produce free radicals); (2) increased release of ferritin under the conditions of decreased cellular high energy compounds; and (3) increased degradation of ATP during hypoxia, increasing the substrate for the xanthine oxidase reaction and leading to increased free radical generation.

135.4.3 Nitric Oxide Free Radicals and Neuronal Injury

The role of nitric oxide (NO) in neuronal injury, both *in vitro* and *in vivo*, has been controversial [42, 43]. This controversy may be due to the use of nonspecific NOS inhibitors. Three major isoforms of NOS have been identified: constitutive neuronal, constitutive endothelial and inducible macrophage isoforms. Following ischemia, NO produced from neuronal NOS has toxic effects, but NO produced from endothelial NOS has protective effects in the brain [44].

Hypoxic brain injury is associated with the formation of NO, a gaseous free radical [45, 46]. Although under normal conditions NO physiologically mediates cerebral vasodilatation, [47] recent studies suggest that NO may react with superoxide anion to form peroxynitrite and cause neurotoxicity [48–51]. Furthermore, *Nw*-nitro-I-arginine (NNLA) a NOS inhibitor, administration in a middle cerebral artery occlusion model reduced the volume of cortical infarct in the mouse, indicating the role of NO in neurotoxicity [52].

Nitric oxide is reported to cause neuronal damage through various mechanisms. We tested the hypothesis that NO synthase inhibition by NNLA will result in decreased oxygen-derived free radical production, leading to the preservation of cell membrane structure and function during cerebral hypoxia [53]. Results demonstrated that free radicals, corresponding to alkoxyl radicals, were induced by hypoxia but were inhibited by pretreatment with NNLA before inducing hypoxia. NNLA also inhibited hypoxia-induced generation of conjugated dienes, products of lipid peroxidation. Na^+, K^+-ATPase activity, an index of cellular membrane function, decreased following hypoxia but was preserved by pretreatment with NNLA. This data demonstrated that during hypoxia NOS generates free radicals via peroxynitrite production, presumably causing lipid peroxidation and membrane dysfunction.

The appearance of primary free radicals, such as superoxide anion or hydroxyl radical, may not indicate oxidative injury. The reactivity of superoxide radicals is limited [54, 55] but hydroxyl radicals are highly reactive to almost all molecules [56] so that they can target even noncritical molecules. Therefore, their concentration does not necessarily correlate with the degree of oxidative damage, particularly when assessing lipid peroxidation. Furthermore, these radicals damage cells in cooperation with other radical species or oxidants [45–48]. In contrast, the production of secondarily formed lipid free radicals provides strong evidence of peroxidative injury. This is particularly true for alkoxyl radicals, which are generated from lipid peroxide by either iron or copper ions and can abstract hydrogen atoms from polyunsaturated fatty acids, leading to further lipid peroxidation [56]. Our results suggested that NO has an *in vivo* role in the generation of alkoxyl radicals, leading to free radical-mediated lipid peroxidation.

The exact molecular mechanism of hypoxic membrane damage is not clear. An appealing hypothesis is that when peroxynitrite (formed by the reaction between superoxide anions and NO) is protonated, it decomposes rapidly to form nitrogen dioxide and hydroxyl radicals, both of which are strong oxidants and can initiate oxidative reactions [45, 48, 57]. It has been shown that peroxynitrite can cause lipid peroxidation *in vitro* [57]. Therefore, high concentration of NO during may result in an increased production of peroxynitrite, causing lipid peroxidation.

135.5 Neuronal Nuclear Ca^{++} influx

A number of critical nuclear functions including regulation of transcription factors, cell cycle regulation transcription, DNA replication and nuclear envelope breakdown are controlled by intracellular Ca^{++} [56, 58]. Furthermore, nuclear Ca^{++} signals potentially control a number of events leading to hypoxia-induced programmed cell death. Nuclear and cytosolic Ca^{++} signals are differently regulated, and the extranuclear Ca^{++} concentration determines the mode of Ca^{++} entry into the nucleus.

The increased intracellular Ca^{++} is a primary mediator of activity-dependent gene transcription under a number of experimental conditions [59–61]. The patterns of neuronal impulse and the specific properties of the stimulus-induced calcium transients determine the nature and amplitude of the genomic response [60, 62]. Several factors including the site of calcium entry, the amplitude and the spatial properties of the calcium signals determine the calcium regulated gene expression [63–65]. Furthermore, the duration of calcium signal also contributes to the specificity of the transcription induction. In cells of the immune system only a continuous rise in intracellular Ca^{++} concentration, but not a brief spike, induced translocation of transcription factors, NF-ATc [66]. Gene expression in neurons is also determined by the duration of

calcium transients and the activity-dependent transcription is regulated by the duration of calcium transients [61].

In previous studies we have shown that cerebral hypoxia results in increased nuclear Ca^{++} influx in neuronal nuclei of the cerebral cortex of newborn piglets [58, 67]. The nuclear Ca^{++} influx increased as a function of increase in cerebral tissue hypoxia, as measured by decrease in high energy phosphates, ATP and phosphocreatine. Cerebral hypoxia results in increased Ca^{++}/calmodulin kinase (CaM kinase) IV activity and CREB protein phosphorylation in neuronal nuclei of newborn piglets [68]. NO donors increased neuronal nuclear Ca^{++} influx [69] and hypoxia resulted in generation of NO free radicals and increased high affinity Ca^{++}-ATPase activity in neuronal nuclei. The high affinity Ca^{++}-ATPase activity increased as a function of increase in cerebral tissue hypoxia [70]. In addition, IP3-dependent Ca^{++} influx is increased in neuronal nuclei of hypoxic animals as compared to normoxic ones and this increase was a function of cerebral tissue hypoxia.

During hypoxia, NO-mediated modification of the nuclear membrane high affinity Ca^{++}-ATPase and IP3 receptor is a potential mechanisms of increased intranuclear Ca^{++} that leads to activation of Ca^{++}-dependent nuclear mechanisms and activates cascades of hypoxic programmed cell death.

135.6 Expression and Post-Translational Modification of Apoptotic Proteins

Bcl-2 family of proteins (including Bc1-2 and Bax) control cell proliferation, differentiation, and programmed cell death during normal brain development [71]. Bax and Bcl-2 are inducible genes found in the developing and adult central and peripheral nervous systems [72, 73]. Bcl-2 prevents apoptosis by forming a heterodimer with the pro-apoptotic protein Bax and protects cells from programmed cell death following hypoxia [71].

Cerebral hypoxia results in increased expression of Bax protein in neuronal nuclei of the cerebral cortex of newborn piglets. The Bax protein increased as a function of increase in degree of cerebral tissue hypoxia as measured by decrease in high energy phosphates, ATP and phosphocreatine [74]. The expression of Bax protein increases in the mitochondrial, cytosolic as well as in the neuronal nuclear fractions indicating increased expression of the protein rather than its translocation e.g., from mitochondria to cytosol. The expression of anti-apoptotic protein Bax to antiapototic protein Bcl-2 did not increase during hypoxia. Therefore, the ratio of proapoptotic protein Bax to antiapototic protein Bcl-2 increases in all compartments of the cell during hypoxia that may lead to activation of hypoxia-induced cascade of neuronal death.

Administration of NOS inhibitor prevented the hypoxia-induced increased expression of proapoptotic protein Bax indicating that the hypoxia-induced increased expression of Bax is NO-mediated [75].

135.6.1 Posthypoxic Expression and Activation of Caspases-3, -8, and 9 in the Newborn Brain

Caspases are a unique family of proteases that play an important role in the initiation and execution of apoptosis [76]. All caspases contain a cysteine residue at their active site and specifically cleave substrate proteins at an aspartic acid residue. These cysteine proteases reside predominantly in the cytosolic compartment of animal cells as inactive zymogens and become activated by proteolytic cleavage at internal aspartate residues on apoptotic stimulation [77]. These are divided into two main classes: those with long prodomain are the Class I caspases, and those with short prodomain are the Class II caspases.

Class I caspases such as 8, 9, and 10 can autocatalyze their own activation and are activated in the early phase of apoptosis. These are called the initiator caspases. Class II caspases, such as 3, 6, and 7 require cleavage by another protease and are responsible for the breakdown of cells [76]. These are called the effector or the executioner caspases. The upstream caspases activated during apoptosis lead to the activation of downstream caspases in a self-amplifying cascade [76].

Two well-studied pathways of caspase activation are the cell surface death receptor-mediated pathway and the mitochondria-initiated pathways. The recruitment and the cleavage of procaspase-8 to produce the active form of caspase-8 is a critical biochemical event in the death receptor-mediated apoptosis [76]. Following its activation, caspase-8 can activate downstream caspase by direct cleavage or indirectly by cleaving the proapoptotic protein and inducing cytochrome c (cyt c) release from the mitochondria [76]. In the proposed mitochondria-initiated pathway, caspase activation is triggered by formation of an oligomeric apoptotic protease activation factor (Apaf-1)/ cyt c complex, which leads in recruiting and activating procaspase-9 an upstream caspase in this pathway [78]. The complex formed by the combination of Apaf-1, cyt c, Bax/Bcl-2, and procaspase-9 is referred to as the apoptosome [78, 79].

Two other less defined pathways of apoptotic caspase activation are the ceramide pathway that may act predominantly through the initiator caspases-8 or -9, depending on the stimulus that induces ceramide synthesis and the granzyme B-perforin pathway, which may be directly initiated by the effector caspases-3 and -7. All these pathways, with the exception of the granzyme pathway, are known to be active in neurons.

Studies were conducted to investigate the role of caspase-cascade-mediated programmed cell death. Using our 3- to 5-day old newborn piglet model, we investigated the hypoxia-induced alterations in the activity and expression of caspase-3, caspase-8, and caspase-9. Results of these studies after 1 hour of severe hypoxia in newborn piglets demonstrated that following hypoxia there is an increase in the activity of the initiator caspase-8 and caspase-9 in the cytosolic fraction of

the cerebral cortex. The activity of caspase-3 also increased in the cytosolic fraction of the cerebral cortex. In addition, the expression of caspase-8, caspase-9, and caspase-3 protein increased following hypoxia [76, 78, 79].

135.6.2 Mechanisms of Caspase Activation During Hypoxia in the Newborn Brain

To investigate the mechanism of caspase activation we used selective inhibitors such as clonidine an inhibitor of high-affinity Ca^{++}-ATPase, 7-nitro-indazole-sodium salt (7-NINA) inhibitor of neuronal NO synthase and Z-leu-Glu (OME) His-Asp (OME)-femomethyl Ketone (Z-LFHD-FMK), a selective caspase inhibitor.

135.6.2.1 The Role of Nuclear Ca^{++} influx in Caspase-9 and Caspase-3 Activation

Studies performed in newborn piglets were specifically designed to investigate the role of nuclear Ca^{++} influx in caspase activation during hypoxia by administration of a Ca^{++}-ATPase inhibitor (clonidine) to block the nuclear Ca^{++} influx. It was shown that the increased activity of casapse-9 and caspase-3 during hypoxia is mediated by nuclear Ca^{++} influx. The levels of tissue high-energy phosphates (ATP and PCr) in the cerebral cortex of normoxic-, hypoxic-, and hypoxic-pretreated with clonidine piglets were comparably decreased. The determination of caspase-9 activity in the normoxic-, hypoxic, and hypoxic-pretreated with clonidine groups of piglets showed that cerebral tissue hypoxia results in increased caspase-9 activity and that the pretreatment with high affinity Ca^{++}-ATPase inhibitor prevents this hypoxia-induced increase in caspase-9 activity.

Assessment of the activity of caspase-3 in the normoxic, hypoxic, and hypoxic pretreated with clonidine groups of piglets demonstrated that cerebral tissue hypoxia resulted in increased caspase-3 activity, a consequence of caspase-9 activation, and that the pretreatment with clonidine prevented this increase in caspase-3 activity. These results demonstrate that hypoxia-induced increase in caspase-3 activity is mediated by nuclear Ca^{++} influx.

135.6.2.2 The Role of NO in Caspase-9 and Caspase-3 Activation

These studies in newborn piglets were specifically designed to investigate the role of NO-derived from neuronal nNOS in caspase activation during hypoxia by administration of a relatively selective inhibitor of nNOS, 7-NINA salt [80]. After having confirmed that cerebral tissue hypoxia achieved in the hypoxic and hypoxic pretreated with 7-NINA groups were comparable, the activity of caspase-9 in the normoxic, hypoxic, and hypoxic-pretreated with 7-NINA groups of piglets was determined, the results demonstrated that cerebral tissue hypoxia results in increased caspase-9 activity and that the pretreatment with the nNOS inhibitor prevents the hypoxia-induced increase in caspase-9 activity.

Cerebral tissue hypoxia resulted in increased caspase-3 activity, a consequence of caspase-9 activation and the pretreatment with the nNOS inhibitor, prevented this hypoxia-induced increase in caspase-3 activity. It demonstrates that the hypoxia-induced increase in caspase-9 is mediated by nNOS-derived NO.

135.6.2.3 The Effect of Caspase-9 Inhibition During Hypoxia on Prevention of Downstream Events Including Caspase-3 Activation

To demonstrate that caspase-3 activation is a downstream event of caspase-9 activation during hypoxia we used its selective inhibitor Z-LEHD-FMK [81, 82].

The activity of caspase-9 was determined in the cytosolic fraction of the cerebral cortex of newborn piglets. Cerebral tissue hypoxia resulted in increased caspase-9 activity and the pretreatment with the caspase-9 inhibitor prevented the hypoxia-induced increase in caspase-9 activity.

The activity of caspase-3 in the cytosolic fraction of the cerebral cortex increased as a consequence of caspase-9 activation, and the pretreatment with a caspase-9 inhibitor prevented this hypoxia-induced increase. The same study showed that pretreatment with caspase-9 inhibitor prevents the hypoxia-induced increase in the expression of active caspase-9 and active caspase-3 (protein levels).

These studies demonstrate that caspase-9 can be activated during hypoxia by multiple mechanisms that are dependent on generation of nNOS-derived NO and neuronal nuclear Ca^{++} influx. The increase in nuclear Ca^{++} influx leading to Ca^{++}-dependent activation of Ca/Calmodulin-dependent protein kinase IV may result in increased phosphorylation or cyclic AMP-response element binding protein at Serine133 and transcription of caspases as well as proapoptotic proteins. NO-mediated phosphorylation of anti-apoptotic proteins may alter their anti-apoptotic potential due to a defect in dimerization and increased caspase-9 activation.

We have shown that NO increases Ca^{++} influx in synaptosomes as well as neuronal nuclei. By increasing nuclear Ca^{++} influx, NO can increase expression of caspase-9 as well as proapoptotic proteins. NO-mediated modification of caspase protein may alter its activation.

Thus, caspase activation during hypoxia in the newborn brain is mediated by transcription-dependent and transcription-independent mechanisms.

Studies using LEHD-FMK, a selective inhibitor of caspase-9, indicate that the role of caspase-9, the inhibitor caspase, is highly significant in the hypoxia-induced programmed cell death in the newborn brain.

135.7 DNA Fragmentation

It has been proposed that the cleavage of DNA at its intranucleosomal linkage region is produced by specific endonucleases that are Ca^{++}-dependent [83].

Caspase-3 acting as cystein protease cleaves and inactivates a chain reaction by nuclear enzymes like PARP, a DNA repair enzyme, and ICAD the inhibitor of caspase-activated DNase. Then the caspase-activated DNase enters the nucleus and cleaves genomic chromosomal DNA [84, 85]. This nuclear genomic DNA fragmentation correlates exponentially with the degree of cerebral tissue hypoxia in newborn piglets [86] and is characteristic of cellular apoptosis. However, in our study, there was no significant increase in fragmentation until the ATP and phosphocreatine levels decreased by more than 50%, compared with baseline levels.

135.8 Temporal Biochemical Changes

The steps of post-hypoxic neuronal injury are evolving within hours in the necrotic process and within days in the apoptotic process. Temporal biochemical changes and associated nuclear fragmentation have been assessed in the cerebral cortex of newborn guinea pigs following hypoxia. Initial cellular injury may be followed by a failure of cellular repair mechanisms leading to further delayed brain injury. Neuronal nuclear Ca^{++} influx increases immediately following hypoxia and remains elevated through 7 days of age. Similar nuclear Bax protein expression increases immediately following hypoxia and remains elevated through 7 days where Bcl-2 protein remains similar to control during hypoxia.

All these temporal (biphasic) temporal changes may reflect not only the primary hypoxic insult but also a secondary cellular damage due to a recurrent (continuing) free radical release during the reperfusion-reoxygenation phase.

135.9 Clinical Implications

The understanding of the very complex and interrelated mechanisms of cell death after a hypoxic-ischemic result may serve as background in critical care of the newborn.

Hypoxia at the cellular level is the consequence of failure in oxygen transport from the lung alveolar space to the mitochondria. In order to prevent neuronal cell death to restore any insufficiency or failure in the respiratory and circulatory systems is an emergency in terms of minutes.

Amongst the hazards in restoring oxygen supply, medically induced hyperoxia (high FIO_2) may worsen the neuronal insult by the production of additional oxygen free radicals.

The temporal evolution of the post-hypoxic biochemical disturbances may alter the opportunity of pharmacologic interventions at key steps of the biochemical events, like magnesium sulfate, a NMDA receptor antagonist, or allopurinol an inhibitor of the enzyme xanthine oxidase.

135.10 Neuroprotective Treatments with Suggested Efficacy in Humans

135.10.1 Magnesium Sulfate

In the clinical setting, $MgSO_4$ has been widely used in obstetrics practice for more than 60 years. Its indications include suppression of preterm labor and management of pregnancy-induced hypertension [87]. A retrospective epidemiologic study by Nelson and Grether [88] suggested that premature fetuses whose mothers received $MgSO_4$ for the treatment of preclampsia or as a tocolytic agent are less likely to develop cerebral palsy compared to a gestational age-matched group of fetuses not exposed to the drug. The Collaborative Eclampsia Trial [89] reported that babies of women who had been given $MgSO_4$ before delivery were significantly less likely to be intubated at the place of delivery or to be admitted to a special care nursery than the babies of mother who had been given phenytoin. These studies suggested that $MgSO_4$ might provide a protective effect against brain damage in immature fetuses and newborn infants. Randomized, controlled, double-blind trials were established to examine this hypothesis. One was discontinued after interim analysis showed that administration of $MgSO_4$ to mothers in preterm labor before 34 weeks of gestation was associated with significant increase in infants' mortality [90]. However, other trials have not shown any difference in the mortality rates between the placebo and treatment groups [91].

A multicenter randomized, controlled trial of $MgSO_4$ versus placebo, for the prevention of cerebral palsy, in 2,241 women at risk of imminent of premature delivery at 24–31 weeks of gestation carried out in the US was published in 2008 [92]. The primary outcome was the composite of stillbirth or infant death by 1 year of corrected age or moderate or severe cerebral palsy at or beyond 2 years of corrected age. The primary outcome was not significantly different in the $MgSO_4$ group and the placebo group. However, in a prespecified secondary anlaysis, moderate or sever cerebral palsy occurred significantly less frequently in the $MgSO_4$ group (1.9% *vs* 3.5%). A similar study in France followed-up 606 infants of less than 33 weeks of gestation, whose mothers were treated with $MgSO_4$. Compared to placebo, treated infants showed a decrease of all primary end points (total mortality, sever white matter injury and their combined outcome) and of all secondary endpoints (motor dysfunction, cerebral palsy, cognitive dysfunction and their combined outcomes at 2 years of age). The decrease was nearly significant or significant for gross motor dysfunction, and combined criteria: death and

cerebral palsy, death and gross motor dysfunction, death, cerebral palsy and cognitive dysfunction [93]. Doyle et al [94] reviewed the evidence of the neuroprotective effects of $MgSO_4$ given to women considered at risk of preterm birth. The authors concluded that the neuroprotective role for antenatal $MgSO_4$ therapy given to mothers at such risk is now established. The number of women needed to treat to benefit one baby by avoiding cerebral palsy is 63 (95% confidence interval 43 to 87). Given the beneficial effects of $MgSO_4$ on substantial gross motor function in early childhood, outcomes later in childhood should be evaluated to determine the presence of absence of later potentially important neurologic effects, particularly on motor or cognitive function.

135.10.2 Allopurinol

In experimental animal models, administration of allopurinol to immature rats 30 minutes before inducing focal hypoxia-ischemia reduced the severity of the secondary edema and the extent of the neuropathologic lesions in the treated compared with a control group [95]. Similarly, pretreatment with allopurinol preserved cerebral energy metabolism of the 7-day postnatal rat during hypoxia-ischemia [96]. The same group of researchers found that oxypurinol, the active metabolite of allopurinol, administered at the same dose and at the same time as allopurinol after hypoxia-ischemia reduced brain injury in the immature rat [97]. Administration of allopurinol in newborn piglets prevented the hypoxia-induced modification of NMDA receptor as well as cell membrane preoxidation and neuronal dysfunction [98, 99].

In the clinical setting, a 7-day course of enteral allopurinol (20 mg per kg) given after birth to 400 infants between 24 and 32 weeks' gestation did not change the incidence of periventricular leukomalacia [100]. In a study of 22 asphyxiated newborn infants, intravaneous allopurinol in a dose of 40 mg per kg given 4 hours after birth resulted in a decrease mortality (2/11 *vs* 6/11 in the control group), and in a beneficial effect on free radical formation, cerebral blood flow, and electrical brain activity, without toxic side effects [101]. Clancy et al [102] conducted a clinical trial to test the hypothesis that allopurinol could reduce death, seizures, coma, and cardiac events in infants who underwent heart surgery using deep hypothermic circulatory arrest. They studied a total of 318 infants, 131 hypoplastic left heart syndrome (HLHS) and 187 non-HLHS. In HLHS surgical survivors, 40 of 47 (85%) allopurinol-treated infants did not experience any endpoint event, compared to 27 of 49 (55%) controls ($p = 0.002$). There were fewer seizure-only ($p = 0.05$) and cardiac-only ($p = 0.03$) events in the allopurinol versus placebo groups. Allopurinol did not reduce efficacy endpoint events in non-HLHS infants. Treated and control infants did not differ in adverse events. Recently, Benders et al [103] investigated whether postnatal allopurinol would reduce free radical induced reperfusion/reoxygenation injury of the brain in severely asphyxiated neonates. In an interim analysis of a randomized, double blind, placebo controlled study, 32 severely asphyxiated infants were given allopurinol or a vehicle within four hours of birth. The analysis showed an unaltered (high) mortality and morbidity in infants treated with allopurinol. The authors concluded that allopurinol treatment started postnatally was too late to reduce the early reperfusion-induced free radical surge. Allopurinol administration to the fetus with (imminent) hypoxia via the mother during labor may be more effective in reducing free radical induced post-asphyxial brain damage.

Chaudhari and McGuire [104] performed a meta-analysis to evaluate the evidence of the effect of allopurinol on mortality or morbidity in newborn infants with suspected hypoxic-ischemic encephalopathy. The authors concluded that the available data are not sufficient to determine whether allopurinol has clinically important benefits for newborn infants with hypoxic-ischemic encephalopathy and, therefore, larger trials are needed. Such trials could assess allopurinol as an adjunct to therapeutic hypothermia in infants with moderate and severe encephalopathy and should be designed to exclude clinically important effects on mortality and adverse long-term neurodevelopmental outcomes.

135.10.3 Opioids

The antinociceptive effects of opioids are mediated through a combination of pre- and postsynaptic hyperpolarization, which produces a decrease in the release of and the sensitivity to endogenous mediators like glutamate [105, 106]. This suggests that they may have a neuroprotective effect. Indeed, studies in cell cultures have demonstrated that endogenous and exogenous opioids may protect cortical neurons from hypoxia-induced cell death [107, 108]. Similarly, opioids may induce ischemic tolerance in cerebellar Purkinje cells subject to ischemia-reperfusion [109]. Antagonists of opioid receptors increase the survival time during severe hypoxia in intact animals [110, 111] and enhance tissue preservation and survival time of organs used for transplants [112].

In 2005, Angeles et al [113] published the results of a retrospective study of 52 term newborns with perinatal asphyxia, in which they analyzed the relationship between treatment with opioid analgesics (morphine or fentanyl) and neurological damage. A total of 33% of them received opioids; in spite of having a more severe degree of asphyxia (higher levels of lactate, lower 5 minute Apgar scores), this group of patients had less severe signs of brain damage on the MRI performed after 7 days of life. Moreover, their neurologic outcome at a mean follow-up of 13 months was better than the group of newborns who did not receive opioids. The same group of researchers also performed a follow-up study with magnetic resonance (MR) spectroscopy of 28 term newborns treated

with opioids and 20 controls [114]. The results showed that occipital gray matter NAA/Cr was significantly decreased and pactate was present in a significantly higher amount in non-opioid-treated neonates compared with opioid-treated neonates. Also, compared with controls, untreated neonates showed large changes in more metabolites in basal ganglia, thalami, and occipital gray matter with greater significance than treated neonates. The authors concluded that the use of opioids during the first week following perinatal asphyxia has no long-term adverse effects, and may increase brain resistance to hypoxia-ischemia. The authors speculated that the neuroprotective effect of opioids may be mediated by increasing the levels of adenosine, and endogenous nucleoside with neuroprotective activity, or by inducing neuronal hyperpolarization, which results in diminishing intracellular penetration of calcium.

Despite the potential benefit of opioids on asphyxiated term neonates as indicated in these studies, caution must be exercised in the use of this class of medications. Available literature suggests that the routine use of opioid analgesics can be complicated by problems such as tolerance, withdrawal symptoms and ventilator dependence. Very few studies have examined the long-term effects of exposure to opioids in the neonatal period. In addition, previous reports indicate that endogenous opioids can suppress DNA synthesis *in vivo* in mature cerebellar and glial cells (opioid receptors are widely distributed in the CNS with functions that include pain modulation, cardiorespiratory regulation), whereas exogenous opioids can exacerbate neurotoxicity in animal models of cerebral ischemia. Future prospective randomized trials are warranted to determine whether there is truly an immediate neuroprotective effect on hypoxic-ischemic brain injury and whether these agents can play a role in improving long-term outcome.

135.10.4 Hypothermia

Hypothermia has developed during the past few yeas as an alternative for treating perinatal asphyxia/HIE [115, 116]. Hypothermia during experimental cerebral ischemia is associated with potent dose-related, long-lasting neuroprotection. Conversely, hyperthermia of only 1–2°C extends and markedly worsens damage, and in particular tends to promote pannecrosis [115]. Although the majority of such studies involved global ischemia in adult rodents [117], similar results were reported from studies on hypoxia-ischemia in 7-day old rats [118] and newborn piglets [119], kittens, rabbits, and puppies [120].

The study of the mechanisms of action of hypothermic neuroprotection suggests that cooling affects many or all of the pathways leading to delayed cell death [115]. Hypothermia reduces the rate of oxygen-requiring enzymatic reactions and cerebral oxygen consumption, slows the fall of phosphocreatine/inorganic phosphate (PCr/Pi), and confers a protective effect of the brain after ATP exhaustion. In addition, hypothermia decreases oxygen consumption of the brain by 6–7% and cerebral energy utilization rate by 5.3% per degree. Additional experimental evidence suggests that hypothermia suppresses cytotoxic excitatory amino acid accumulation, inhibits nitric oxide synthase activity, decreases interleukin-1 levels, decreases the release of other cytotoxic cytokines by microglial cells, and suppresses free radical activity and delayed cell death by apoptosis. Hypothermia also decreases blood-brain-barrier permeability and intracranial pressure, and facilitates recovery of electrophysiologic function after cerebral ischemia.

The efficacy of hypothermia is dependent on a number of factors, such as the timing of initiation of cooling, its duration, and the depth of cooling attained. Mild hypothermia is defined as a reduction in core temperature of 1–3°C, moderate as 4–6°C, severe as 8–10°C and profound as 15–20°C. Brief (0.5–3 hours), mild-to-moderate hypothermia immediately after hypoxia-ischemic injury may be most effective after relatively mild insults. Protection appears to be lost if brief hypothermia is delayed by as little as 15–45 minutes after the primary insult. A more recent approach has been to try to suppress the secondary encephalopathic processes by maintaining hypothermia throughout the course of the secondary phase. An extended period of cooling (between 5 and 72 hours) appears to be more consistently effective, and remains effective after significant delays (possibly up to 6 hours) between the primary insult and the start of cooling; however, the degree of neuroprotection progressively declines if cooling is initiated more than a few hours post-insult [115]. In addition, cerebral hypothermia is not neuroprotective when started after post-ischemic seizures occur [121].

Potential adverse effects of induced hypothermia (the risk increasing with depth of hypothermia) include increased blood viscosity, mild metabolic acidosis, decreased oxygen availability, intracellular shift of potassium, cardiac arrhythmias, coagulation abnormalities and platelet dysfunction, and choreic syndrome [116].

135.10.5 Selective Head Cooling

The first study on neuroprotection of perinatal HIE with selective head cooling was published in 1998 by Gunn et al [122], who basically proved the safety of this procedure. Later on, the same group of researchers published the results of other studies in a small number of patients, which confirmed the lack of side effects and a tendency to a better neurologic prognosis in those newborns with moderate or severe HIE treated with this hypothermia technique [123, 124].

In 2005 Gluckman et al [125] published the results of the most important study that has produced reliable and significant data about the neuroprotective effect of selective head cooling hypothermia. It was a multicenter investigation that

included 234 newborns with HIE and expected gestational age (EGA) above 36 weeks. Patients were randomized before 5.5 hours of life into two groups: body normothermia or hyporthermia of 34.5°C, induced through selective head cooling during 72 hours. Patients treated with hypothermia had a significantly higher incidence of arrhythmia (mostly sinus bradycardia). A total of 218 infants were followed-up until 18 months of age. The presence of death or neurological disability was found in 66% of patients in the control group and 55% in the hypothermia group ($p < 0.1$). However, when newborns with severe neurological depression or those who had seizures on the aEEG were excluded, 66% of infants in the control group and 48% in the hypothermia group died or had neurological disability ($p < 0.2$). Moreover, the presence of severe neurological disability was 28% and 12% in each group, respectively. The authors concluded that, except in newborns with the most severe forms of HIE, selective head cooling applied immediately following delivery, may be a feasible therapeutic technique to decrease neurological sequelae of perinatal HIE.

135.10.6 Generalized Body Hypothermia

The first study with generalized body hypothermia in perinatal HIE was published in 2000 by Azzopardi et al [126], who found that prolonged hypothermia of 33–34°C was associated with minimal physiological changes (e.g., decreased heart rate, increased blood pressure), but was well tolerated. During the next three years, other research protocols in a limited number of patients corroborated that generalized body hypothermia was a feasible and clinically safe technique [127, 128].

In 2005, Eicher et al [129] published the results of a pilot multicenter study about the safety and efficacy of generalized body hypothermia in the treatment of 32 newborns with perinatal HIE. Adverse effects included bradycardia, hypotension, decreased platelets, increased prothrombin time, and higher incidence of seizures, but none of them was severe, and they all responded to treatment [129]. The efficacy results showed a higher incidence of death or severe neurological motor involvement in the control group (82%) compared to the group of hypothermia-treated newborns (52%) ($p = 0.019$). A severe psychomotor developmental delay (less than 70%) was seen in 64% of infants in the control group and in 24% of those subjected to hypothermia ($p = 0.053$).

Also in 2005, Shankaran et al [130] published a large multicenter study on the use of body hypothermia to treat perinatal HIE. A total of 208 newborns with HIE and EGA more than 36 weeks, were included and randomized before 6 hours of life into two groups: body normothermia or hypothermia of 33.5°C, induced by body cooling, during 72 hours. Patients were followed-up until 18–22 months. The incidence of mild complications was similar in both groups. The incidence of death or moderate or severe neurological disability was 62% in the control group and 44% in the hypothermia treated group ($p = 0.01$). The incidence of cerebral palsy was 30% in the control newborns and 19% in those treated with hypothermia ($p = 0.20$). The authors concluded that generalized body hypothermia reduces the risk of death and neurological disability in newborns with moderate or severe HIE.

An magnetic resonance imaging (MRI) follow-up study of infants enrolled in the above mentioned trial [130] aimed to measure relative volumes of subcortical white matter. They were significantly large in hypothermia-treated than in control infants. Furthermore, relative total brain volumes correlated significantly with death or neurosensory impairments. Relative volumes of the cortical gray and subcortical white matter also correlated significantly with Bayley Scales psychomotor development index [131].

Hypothermia is currently in the process of translating from the clinical research experience to direct clinical application [132–141]. However, the clinical trials with hypothermia have also addressed many questions, which need to be answered before it becomes the standard of care to treat newborns with perinatal asphyxia.

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Clinical Aspects and Treatment of the Hypoxic-Ischemic Syndrome

Floris Groenendaal and Frank van Bel

136.1 Epidemiology

In the Western world, perinatal asphyxia is still a relatively common phenomenon in perinatal care. Since differences in causes and patterns of brain injury following perinatal asphyxia exist between full-term and preterm neonates, this chapter will focus on full-term neonates.

Generally, it has been assumed that in the Western world 0.1–0.4 % of all full-term neonates need resuscitation at birth because of perinatal asphyxia [1, 2]. Recent studies indicated that at least 0.5% of full-term neonates needed some degree of resuscitation at birth because of a 5-minute Apgar score of 6 or less [3]. Of all full-term neonates, 0.07% had a 5-minute Apgar score of 3 or less. Mortality in these severely depressed neonates was 24%.

Data from the Netherlands Perinatal Registry show that at least 0.1% of all full-term neonates develop encephalopathy after severe perinatal asphyxia.

Although the concept of perinatal asphyxia has been challenged by some, a recent combined magnetic resonance imaging (MRI) and pathology study showed recent hypoxic-ischemic changes in brain tissue in 80% of full-term neonates with perinatal asphyxia and encephalopathy [4].

Perinatal asphyxia may be caused by antenatal factors, such as severe maternal diseases or shock, perinatal factors such as a difficult delivery with compromised blood flow through the umbilical cord, placental abruption or uterine rupture, or postnatal factors such as respiratory difficulties as in congenital diaphragmatic hernia or muscle diseases. The distribution of antenatal, perinatal or postnatal factors in the causation of asphyxia is largely unknown.

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136.2 Pathology and Outcome

During perinatal asphyxia two different processes contribute to brain injury: hypoxia (lack of oxygen) as well as ischemia (lack of blood flow due to bradycardia, thereby reducing flow of e.g., glucose) contribute to cellular compromise. This is even further aggravated by reperfusion with production of a large amount of oxygen and nitrogen free radicals and non-protein bound iron. Brain injury is the resultant of hypoxia-ischemia and reperfusion.

Different patterns of cerebral involvement after perinatal asphyxia have been described in post mortem studies, and have been confirmed in experimental animals and *in vivo* in the human neonate using MRI:

1. Total brain necrosis
2. Injury of basal ganglia and thalamus
3. Watershed lesions

Total brain necrosis is the most serious form of brain injury. Most infants with this lesion will not survive, since the brain stem is involved in the pathology. Pathology studies have described this pattern in detail. In the rare neonate who survives, multicystic encephalomalacia or ulegyria can be seen [5]. These children will show major and multiple handicaps, including cerebral visual impairment.

Injury of the basal ganglia and thalamus are seen in neonates with an acute and severe insult with no previous signs of fetal compromise. This pattern has been demonstrated *in vivo* in neonates using cranial ultrasound, and in the last decade using MRI (Fig. 136.1). In many patients injury to the hippocampus, a brain region known to be sensitive to hypoxic-ischemic injury, can be identified using diffusion weighted MRI. In surviving neonates this pattern of brain injury leads to gliosis of the basal ganglia and thalamus. On post mortem examination this pattern has been referred to as status marmoratus because of the marble like appearance [5]. Children with this type of cerebral injury display the athetoid type of cerebral palsy. Epilepsy has been described, possibly due to injury of the hippocampus.

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

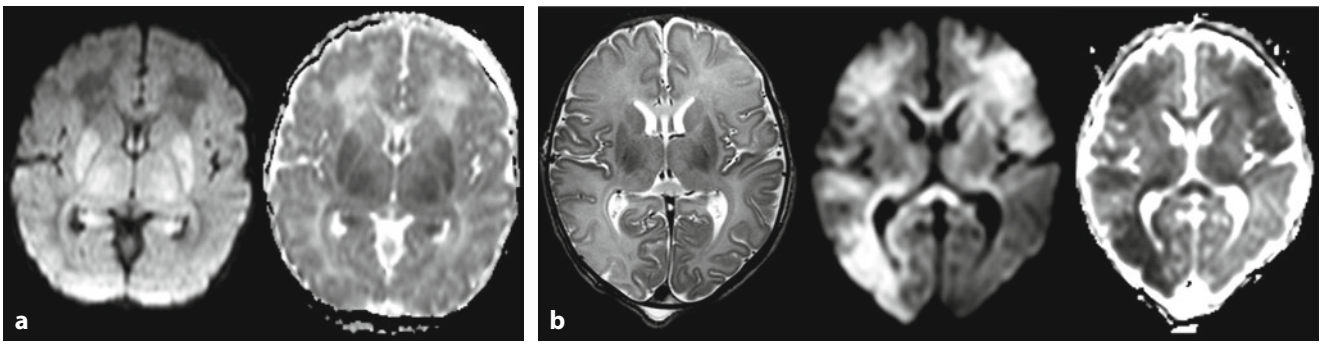

Fig. 136.1 MRI of two full-term neonates with perinatal asphyxia. **a** Abnormalities of the basal ganglia and thalamus on the 4th day of life in a full-term neonate with perinatal asphyxia: DWI (*left*) and ADC map (*right*). ADC values of the basal ganglia were 770×10^{-6} mm^2/s. The patient died 5 days after birth. **b** Abnormalities of gray and white matter in a full-term neonate with watershed infarcts. MRI on day 3 after birth. T2 weighted (*left*), diffusion weighted (*middle*) images, and ADC map (*right*). This infant showed normal development at 1 year of age

Watershed lesions were described in the late 1970s using technetium scans [6, 7]. Reduced perfusion of cerebral border zones between anterior and middle cerebral arteries and middle and posterior cerebral arteries are the most important aspect of this type of lesion. A more chronic, intrauterine form of perinatal asphyxia has been suggested as the causative factor of this type of lesion.

In more severe cases long-term effects are volume loss of the frontal and parieto-occipital parts of the cerebrum, but in milder cases we have observed normal development without long-term effects up to 2 years of age [8]. In more severe cases, disturbances of mental development are the most important long-term effects.

In the present chapter we will not discuss focal infarcts. Focal infarcts have been increasingly demonstrated in full-term as well as preterm neonates. However, since they occur in neonates who in most cases had no signs of perinatal asphyxia, these types of brain lesions are beyond the scope of this chapter.

136.3 Clinical Picture of Hypoxic-Ischemic Syndrome

As can be extracted from the former section discussing the etiology of the hypoxic-ischemic syndrome, the basis of this syndrome is mostly (± 70%) a disturbance in gas exchange between the fetus and mother in the direct antepartum and intrapartum period, at least when referring to the full-term infant. Although there is no ideal measure to assess, from the clinical point of view, the severity of perinatal hypoxia-ischemia during the initial clinical presentation of the infant immediately after birth, the most used instrument here is the Apgar score, introduced in 1953 by the anesthesiologist Virginia Apgar [9]. The Apgar score is usually recorded at 1 and 5 minutes after birth and includes an assessment of heart, pulmonary and neurological functions by an assessment of color, heart rate, respiration and tone (see also below). In 1992 the American Academy of Pediatrics and the American College of Obstetricians Committees on Maternal-Fetal Medicine defined the following criteria for the presence of perinatal asphyxia, consisting out of [10]:

- a metabolic parameter (arterial umbilical cord pH < 7.00);
- a persistence of an Apgar score of 0-to-3 at 5-minutes of extrauterine life;
- clinical and neurological sequelae in the immediate neonatal period;
- clinical and chemical evidence of multi-organ failure in the early neonatal period.

Lactate levels and lactate/pyruvate ratios in arterial umbilical cord blood or neonatal arterial blood in the first hour of life are also indicators of pre-existing substantial perinatal asphyxia [11]. Heavy meconium staining of amniotic fluid before delivery is associated with perinatal asphyxia and a poor clinical condition at birth, and related to early death [12]. We will discuss these issues more in detail under the section dealing with diagnosis and prognosis.

The persistence of clinical symptoms after antepartum- and perinatal hypoxia-ischemia differ and are related to the severity and duration of the actual hypoxic-ischemic insult [13]. Symptoms can range from subtle neurological symptoms such as a transient drowsiness without involvement of other organ systems to overt involvement of important organ systems with renal, cardiac and pulmonary abnormalities in association with a full-blown neurological syndrome [14–17]. We will discuss here those conditions where perinatal hypoxia leads to dysfunctions of the central nervous system and other important organ systems.

During the first 12 to 24 hours of life after moderate-to-severe perinatal fetal hypoxia-ischemia, infants show abnormal neurological behavior which ranges from relatively mild to an overt abnormal neurological picture. Mildly affected infants show increased restlessness and moderate hypertonia

often accompanied by periodic breathing and feeding difficulties, which subside in the first days of life [18]. Part of these symptoms may be related to an abnormal stimulation or transient malfunction of certain regions of the central nervous system.

Severely asphyxiated infants show a variety of neurological symptoms such as a lowered level of consciousness, hypotonia, gazing with abnormal papillary size, eventually with subtle signs of seizure activity (sucking, odd movements of the mouth with smacking etc.). The most severely affected infants are often profound lethargic or even stuporous and show already in an early phase overt convulsive activity as indicated by apneic spells and abnormal (clonic or tonic) movements. In full-term infants these seizures are mostly multifocal with clonic characteristics, whereas less mature infants show often tonic seizures which are often generalized [19–21]. Systemic symptoms may be lacking in the mildly affected infants but mostly there is a decrease or even a lack of urinary production because of a mostly transient hypoxic damage to the kidneys. Renal function recovers almost always unless hypoxic-ischemic damage has been irreversible, which is always related to extensive damage to the brain [22]. In the more severely affected babies there is almost always respiratory insufficiency with prolonged apnea, probably related to malfunction of the central nervous system, sometimes giving rise for the need of artificial ventilation, often somewhat later in life, after 48 to 72 hours of life. An hypoxia-induced myocardiopathy is a prominent feature and its severity mirrors the severity of the perinatal hypoxic-ischemic insult [23, 24]. In most cases, however, there are no or only mild clinical signs, although relatively often inotropes are needed to prevent hypotension [24]. In most infants recovery of myocardial contractility occurs in the first 24 to 48 hours after birth [23]. In a minority of cases massive cardiac failure occurs, but in such situations there is invariably severe irreversible brain damage.

From 24 hours onward, those infants with initially mild-to-moderate neurological symptoms can exhibit an improvement of their clinical and neurological condition with normalization of consciousness and neurological behavior and start to drink their feedings. In these infants also urine production starts or increases and no further support of blood pressure is necessary. This is accompanied by normalization of their plasma parameters of renal (creatinine, urea) and liver functions and of echocardiographically determined cardiac function. Infants who are neurologically normal at one week of life have mostly a normal developmental outcome [25, 26]. Those infants with more severe neurological symptoms, often accompanied with renal and cardiac failure will often deteriorate. The level of consciousness decreases and in this particular time frame respiratory insufficiency becomes manifest and intubation and assisted ventilation is necessary, probably on a neurological basis. Derailment of brain stem functions become manifest now with further loss of responsiveness, overt and increasing frequency of clinical and subclinical seizure activity and abnormal movements of the eyes and dilated pupils [17]. An explanation for this delayed deterioration is probably related to secondary energy failure of the brain which occurs typically between 24 and 72 hours after birth or the hypoxic-ischemic insult [27, 28]. In this particular time frame the most severely affected children die and autopsy invariably shows cytotoxic brain swelling and major and extensive neuronal damage [24]. When these severely asphyxiated infants survive, their physical condition shows a sustained improvement but they will continue to have abnormal neurological features with (slight) disturbances in consciousness, feeding difficulties because of decreased sucking and swallow abnormalities. Dependent of the site of cerebral damage, the infants show overt hypotonia or hypertonia (in particular during involvement of the basal ganglia). Also here it is important to realize that the stage of development of the nervous system gives rise to a different clinical symptom-complex after perinatal hypoxia-ischemia especially in relation to tone and the occurrence of hemiparesis.

It was already years ago suggested that the neurological condition in the first few days of life was a more specific predictor of subsequent neurological outcome than signs of fetal distress or the Apgar score [29].

In 1976 Sarnat and Sarnat [25] therefore carried out a score, the Sarnat score, which was a combination of neurological symptoms as described above and the accompanying electro-encephalographic (EEG) features. This score was in fact a grading of the severity of the encephalopathy caused by the perinatal hypoxia-ischemia. It contains three stages, hypoxic-ischemic encephalopathy (HIE) stage 1, HIE stage 2 and HIE stage 3, the latest the most severe stage of encephalopathy. The most severely affected infants typically progress from stage 1 to 3 (Table 136.1). In 1983 Fenichel modified this score to a score solely on clinical symptoms, which distinguishes a mild, moderate and severe encephalopathy which could be linked to later neurodevelopmental outcome (Table 136.1) [30–32].

The Thompson score [33] is comparable to the Sarnat and Fenichel scores but can be used already shortly after birth, whereas the Sarnat and Fenichel scores can be done earliest at 24 hours after birth. These scores indicate the severity of the hypoxic-ischemic encephalopathy which correlate directly with the incidence of neurological sequelae [34, 35]. Follow-up studies showed that infants with mild hypoxic-ischemic encephalopathy (Sarnat HIE stage 1) did not have subsequent deficits, whereas in infants with moderate encephalopathy, 5% died (Sarnat HIE stage 3), 24% had neurological sequelae and 71% were apparently normal. Of those infants with severe encephalopathy, 80% died and 20% had severe neurological sequelae [17]. In particular the Sarnat score is widely used these days to assess the severity of hypoxia-ischemia-induced encephalopathy and subsequent outcome and to assess the positive impact on outcome of newly designed therapies such as hypothermia and other means of neuroprotection [36–39].

Table 136.1 Hypoxic-ischemic encephalopathy (HIE) according to Sarnat [25] (a) and Fenichel [30] (b)

a. Symptoms	Stage 1	Stage 2	Stage 3
Consciousness	hyperalert	lethargic or obtunded	stuporous
Neuromuscular control	normal	mild hypotonia	flaccid
Suck/Moro reflex	normal	weak	absent
OV/TN reflex	normal	strong	absent
Autonomic function	in general sympathetic	in general parasympathic	both depressed
Seizures	none	(multi)-focal	decerebrated
EEG findings	normal	low voltage (δ/0 waves)	isoelectric
Duration	<24 h	2-14 days	hours to weeks
b. Symptoms	**Mild**	**Moderate**	**Severe**
Consciousness	irritable/hyperalert	lethargic	comatose
Tone	mildly abnormal	moderately abnormal	severely abnormal
Suck reflex	abnormal	poor	absent
Primitive reflexes	exaggerated	depressed	absent
Seizures	absent	present	present
Brain stem reflexes	normal	normal	impaired
Respiration	tachypneic	occasional apnea	severe apnea

OV oculo-vestibular, *TN* tonic neck.

136.4 Treatment of Hypoxic-Ischemic Syndrome

136.4.1 Reduction or Prevention of Fetal Hypoxia

Prevention may be the most important issue when discussing treatment modalities with respect to perinatal hypoxia-ischemia syndrome. Since the primary insult is in a vast majority of cases intrauterine hypoxia, early identification may alter long-term consequences of hypoxia-ischemia syndrome. However, it must be admitted that extensive ante- and intrapartum monitoring of the heart rate of the high risk fetus has led to increased obstetrical interventions without improving neonatal outcome [40], although the combination with fetal blood gas assessment and the introduction of the fetal electrocardiography technique may contribute to a reduction of fetal hypoxia-related long-term adverse outcome [41, 42]. Adequate interventions such as emergency cesarean section may prevent progression from mild fetal hypoxia to a moderate or severe perinatal hypoxic-ischemic syndrome. Although beyond the scope of this chapter, antenatal drug treatment via the mother with free radical scavengers or other neuroprotective agents may be an option to reduce or prevent hypoxic brain damage of the fetus [38, 43].

136.4.2 General Supportive Care

The baby born after substantial perinatal hypoxia-ischemia shows, in addition to nervous system damage, mostly dysfunction of several other important organ systems. In order of occurrence and importance one should mention here: pulmonary dysfunction with insufficient ventilation and oxygenation and cardiac dysfunction with suboptimal or overtly low blood pressure [23, 44]. Later on renal and liver function disturbances and clotting abnormalities and hyperviscosity can occur which need treatment [22, 45]. Details are presented in Table 136.2.

136.4.2.1 Ventilation and Oxygenation

Especially non-adequate ventilation and persistence of hypoxia can further aggravate nervous system damage and

Table 136.2 General supportive care

During resuscitation phase
- Establish heart rate monitoring
- Establish adequate ventilation (if needed: intubation and artificial ventilation)
- Connect infant with pulse oximeter for appropriate oxygenation
- Avoid hyperoxygenation (to avoid free radical production and ROP [preterm baby])
- Avoid hyper-/hypocapnia (preterm baby)
- Correct metabolic acidosis (after normalization of $PaCO_2$)
- Insert intravenous line for fluid and glucose administration

Subcutane phase
- Cardiac support, if necessary, with positive inotropes (hypotension) and/or fluid (blood loss)
- Aim for normoglycemic plasma levels

After stabilization
- Ensure appropriate caloric intake by intravenous route
- First 48 h no enteral feeding
- Monitor urine production
- Monitor coagulation and thrombocytes
- Treat convulsive activity (see also Table 136.3)

should be treated promptly. Although oxygen supplementation is an issue nowadays and hyperoxia during the resuscitation phase has been reported to contribute to additional reperfusion injury to neuronal cells, adequate oxygenation of the compromised newborn is crucial. It becomes more and more clear that this is also possible with room air and that resuscitation with 100% of oxygen is not desirable and damaging [46–48]. In our unit, as soon as possible the high risk infant is connected to the pulse oximeter to monitor systemic arterial saturation in order to avoid hyperoxia and/or hypoxia during the initial phase of resuscitation [49]. It is further important to realize that hypoxia and hypercapnia negatively influence the infant's capability to autoregulate the cerebral vascular bed with, as a consequence, blood pressure passive cerebral perfusion. Therefore assisted ventilation with mask and balloon or after endotracheal intubation and artificial ventilation may be an important option to stabilize the newborn and to correct in a quick and appropriate way the blood gases and metabolic demands. A special remark should be made with regard to hypocapnia, since the effects of hypocapnia are pronounced and have important metabolic and vascular effects [50].

136.4.2.2 Cardiac Dysfunction

Adequate perfusion of the brain is mandatory to prevent additional cerebral damage. Perinatal hypoxia-ischemia has been known to cause hypoxia-induced myocardiopathy which can lead to transient hypotension and hypoperfusion of important organs such as the brain [23]. Especially during severe perinatal hypoxia-ischemia, positive inotropes may be needed to prevent hypotension, although this myocardiopathy subsides mostly within the first 24 to 48 hours of life [23]. Mostly dobutamine is preferred above dopamine because of its positive inotropic effect on the myocardium without the peripheral effects such as peripheral vasoconstriction which may further compromise cardiac function. This is also the reason to avoid volume expanders if possible, although in a number of conditions circulating blood volume can be too low, such as in cases with fetal and neonatal blood loss during the perinatal period and/or delivery.

136.4.2.3 Additional Measures

Normoglycemic levels should be maintained, and adequate protein and caloric intake should be provided as soon as possible. Furthermore it is important to realize that early enteral feeding may facilitate the occurrence of necrotizing enterocolitis, because the gut may be already affected by hypoxic-ischemic damage [51]. We systematically postpone enteral feeding up to 48 hours of life and start as soon as possible after birth parenteral feeding. It is generally accepted that seizure activity, especially when occurring frequently and prolonged, is associated with a high metabolic demand and can, ultimately, adversely affect the brain. Below we will discuss in more detail treatment of seizure activity [52]. Birth asphyxia-related disseminated intravascular coagulation reduces levels of coagulation factors and thrombocytes, which can result in prolonged bleeding times [53]. Renal dysfunction accompanied by acute necrosis of the tubules is often a temporary problem that resolves over the first week of life and needs rarely more specific therapy such as hemodilution [54]. Table 136.2 summarizes the general care measures to be undertaken in the perinatally hypoxic-ischemic infant.

136.4.3 Treatment of Seizures

Since seizure activity has been recognized to be related to add substantially to additional neuronal damage [52], early recognition of seizure activity is extremely important. It therefore recommended to connect the neonate at risk for birth asphyxia-related brain damage to a one or two-channel continuous amplitude-integrated electroencephalography monitor, which facilitates detection of subclinical seizures which frequently occur during the first days after perinatal hypoxia-ischemia [55, 56]. Furthermore seizures can cause sudden increase of blood pressure and hypoglycemia, further aggravating asphyxia-related brain damage [57].

In our institution we use phenobarbital as a first line anticonvulsive with a total dose not exceeding 30 mg/kg iv. If necessary, phenobarbital can be given as maintenance, starting 1 week after the first dose. When seizure activity continues or reappears we use midazolam as a second drug starting with a loading dose followed by a maintenance dose as a continuous infusion (up to 0.5 mg/kg iv). In the baby with a gestational age of less than 35 weeks the second choice is clonazepam with a loading dose and maintenance dose (up to 0.5 mg/kg iv), since midazolam can induce hypotension in the preterm

Table 136.3 Dosing scheme of anticonvulsive drugs used in our institution for full-term neonates

Full-term infant	
• First choice	Phenobarbital: 20 mg/kg iv; if necessary 2nd dose of 10 mg/kg iv
• Second choice	Midazolam (baby CA > 35): – loading dose 0.05 kg in 10 min iv – maintenance dose 0.15-0.5 mg/kg/h continuous iv
• Third choice	Lidocaine*: loading dose 2 mg/kg in 10 min iv 6 mg/kg continuous infusion for 6 h 4 mg/kg continuous infusion for 12 h 2 mg/kg continuous infusion for 12 h
During hypothermia	
Lidocaine: loading dose	
	2 mg/kg in 10 min iv; 4 mg/kg continuous infusion for 6 h 2 mg/kg continuous infusion for 12 h

* A new dose schedule was suggested recently [156].

baby because of its vasodilating effect [58]. If a third antiepileptic drug is necessary, we use a tapering continuous iv infusion of lidocaine. Although the effect of lidocaine can be sustained (after 1 to 1.5 hours) it has a very good antiepileptic activity. During lidocaine treatment the ECG should be monitored because lidocaine can cause cardiac arrhythmia although in our hands and dosing regimen it is extremely rare [59]. If possible we recommend serial determinations of plasma lidocaine concentrations. This is also true when we use it in severely asphyxiated infants on moderate hypothermia, from which it is known that hypothermia influences pharmacokinetics [60]. Table 136.3 provides a detailed dosing scheme of mostly used anticonvulsive drugs in our institution.

136.4.4 Specific Treatment of Perinatal Hypoxic-Ischemic Syndrome

During perinatal hypoxia and concomitant ischemia and especially during reoxygenation and reperfusion at birth, potentially destructive molecular pathways are activated in the fetal and neonatal brain that ultimately lead to brain damage [61]. Animal and clinical studies demonstrated excessive influx of calcium into the neuronal cells through ion-regulated and N-methyl-D-aspartic acid (NMDA)-receptor-regulated channels. This intracellular calcium gives rise to enzyme activation and production of reactive oxygen and nitrogen species which damages neuronal cell membranes and DNA. Moreover, anti-inflammatory and apoptotic activity is activated [62]. Experimental and human studies have shown that the energy metabolism of the perinatally hypoxic-ischemic brain recovers upon reperfusion/reoxygenation, but that after 6 to 12 hours the energy metabolism of the brain becomes increasingly disturbed and irreversible brain damage occur [27, 28, 63]. It is likely that full development of destructive molecular pathways as mentioned above occurs in this time frame and that in the first 6 hours after recovery of brain perfusion and oxygenation (also called the therapeutic window) (pharmacologic) therapies aimed to prevent activation of these destructive molecular pathways may have a neuroprotective effect. This assumption was indeed supported by evidence from experimental studies in several newborn animal species [64, 65]. A more detailed review is beyond the aim of this chapter , but we will briefly summarize those therapies already established in daily practice, such as moderate hypothermia, and those which may be suitable for clinical use in the near future. Moderate hypothermia down to 33–34°C for 72 hours and starting within 6 hours after birth, either selective head cooling [36] or total body cooling [37], has been proven to be neuroprotective after moderate-to-severe perinatal hypoxia-ischemia. A meta-analysis of two large cooling studies showed that especially the infants eventually developing moderate birth-related encephalopathy benefit from this approach [66]. Although hypothermia seems to be a rather safe therapy, adverse effects may occur. During cooling hypertension, pulmonary hypertension, thrombocytopenia and hypokaliemia are reported, whereas during rewarming hypotension may occur [67]. There are preliminary studies which report an altered pharmacokinetic pattern of anticonvulsants and morphine, which are frequently used during this condition [60]. We suggest therefore to confine hypothermia for treatment of neonatal posthypoxic-ischemic encephalopathy to neonatal intensive care units [68].

It is conceivable that combining moderate hypothermia with a pharmacological means of treatment may further improve outcome after perinatal hypoxia-ischemia [65]. It is even possible that fetuses with imminent hypoxia, if timely detected, will receive neuroprotective (pharmacologic) treatment in utero, further ameliorating the consequences of perinatal hypoxia-ischemia. In the Chapter 137 this will be discussed more in detail.

136.5 Diagnosis of the Severity of Perinatal Asphyxia and Prediction of Long-term Outcome

Animal experiments have demonstrated that perinatal asphyxia can lead to brain injury and cerebral palsy comparable to patterns seen in the human neonate [17, 69, 70]. During the last decade many studies have focused on early detection of brain injury in neonates following perinatal asphyxia. Meanwhile, research has focused on neuroprotective strategies aiming at a reduction of permanent brain injury. In this section both assessment of the severity of the hypoxic-ischemic event as well as prediction of long-term outcome will be discussed.

136.5.1 Prenatal Assessment of Fetal Hypoxia

Abnormal fetal heart rate patterns [71, 72], changes in umbilical and cerebral blood flow observed using antenatal Doppler examinations [71, 73], altered fetal behavior [74, 75], abnormal scalp blood gas values [76] or the intrauterine passage of meconium [77] may indicate fetal hypoxia. However, they do

Table 136.4 Indications of fetal hypoxia

Indications of fetal hypoxia	Reference
Fetal heart rate patterns	[71, 72]
Doppler examinations	[71, 73]
Fetal behavior	[74, 75]
Scalp blood gases	[76]
Meconium production	[77]
Fetal ECG changes	[79]

not predict the development of neonatal encephalopathy, and are poor predictors of the development of cerebral palsy [78]. Recently, changes in the fetal electrocardiogram (ECG) have been reported to correlate well with fetal hypoxia. This so-called STAN method needs further study, but appears a promising tool for better evaluation of the fetus (Table 136.4) [79].

136.5.2 Postnatal Assessment of the Neonate

136.5.2.1 Apgar Score

Virginia Apgar has published a scoring systems for clinical assessment of the neonate [9]. This scoring system has been designed to help in the decision whether or not to resuscitate the neonate, but has been wrongfully used to make the diagnosis of perinatal asphyxia [80].

Since the Apgar score can be low due to transplacentally obtained drugs that depress the central nervous system (like anesthesia), trauma during delivery or reflex inhibition of breathing due to frequent suctioning, clinicians debated whether a low Apgar score always reflects perinatal asphyxia, and predicts neonatal encephalopathy and cerebral palsy. Nevertheless a 10 min Apgar score of 5 or less had a sensitivity of 43% and a specificity of 95% towards an adverse outcome [31]. A good Apgar score does not guarantee the absence of cerebral palsy (CP) in later life, since neonates with antenatally acquired brain lesions may have normal Apgar scores [81]. Apgar scores of 0–3 at one minute were found in 31% of full-term neonates with encephalopathy compared to 1% of control infants [82]. In neonates with seizures Apgar scores were significantly lower at 1–20 minutes after birth compared to control neonates [83].

In preterm infants the Apgar score may be influenced by prematurity [84]. Respiratory efforts, muscle tone, and reflex activity were significantly lower at declining gestational ages. The Apgar score alone is not enough to ascertain perinatal asphyxia and predict neonatal encephalopathy.

136.5.2.2 Blood Gas Values

In the years following the use of the Apgar score insight was obtained in the values of pH, oxygen and carbon dioxide in fetal scalp blood [76] and umbilical cord blood [85]. It was shown that severely depressed neonates had lower pH values, larger base deficits and elevated levels of lactate compared to vigorous and moderately depressed neonates, reflecting anaerobic glycolysis [85]. Sykes et al demonstrated that Apgar scores do not reflect the degree of acidosis at delivery [86], as only 19% of the neonates with a 5 min Apgar score < 7 had a pH ≤ 7.10 and a base deficit ≥ 13 mmol/L and 86% of the neonates with a pH ≤ 7.10 and a base deficit ≥ 13 mmol/L had a 5 min Apgar score ≥ 7. Similar findings were reported thereafter [87]. A pH value below 7.0 seems to have a better predictive value towards a poor outcome than higher cut-off values [15, 88, 89]. In the study by Goodwin et al, 31% of the term neonates with a pH of the umbilical artery below 7.0 developed neonatal encephalopathy. Neonates with more severe forms of acidosis had a much higher chance of seizures and more severe forms of neonatal encephalopathy [15]. Recently, this was confirmed in our institute. In addition, the good outcome in the encephalopathic neonates suggests that an adverse long-term outcome could be prevented with adequate neonatal care (Table 136.5) [90].

Table 136.5 Postnatal evidence of asphyxia

Postnatal evidence of asphyxia	Reference
• Apgar score	[9, 80]
• (Cord) Blood Gas Values	[15, 76, 85, 87–92]
• Lactate, Hypoxanthine	[11, 87, 100–102, 105]
• Brain Specific Proteins	[108–110]
• Cytokines	[113–118]
• Clinical Scoring Systems	[25, 121, 33, 122]
• Neurophysiology	
– Evoked Potentials	[150–155]
– aEEG	[56, 143–145, 147, 149]
• Neuroimaging	
– CBF	[32, 124]
– NIRS	[123, 125]
– Ultrasound	[126]
– MRI	[4, 8, 127–134]
– MRS	[135–137]

Others have used a low buffer base in the umbilical artery as a sign of fetal hypoxia. Of the 25 neonates with a buffer base < 30 mmol/L, 14 (56%) developed neonatal encephalopathy, and only six (2%) severe neonatal encephalopathy [91]. Nevertheless, unexpectedly low pH values in vigorous neonates do not seem to result in neonatal encephalopathy [92]. Elevated levels of (nor)adrenalin may play a role in these hypoxic but vigorous neonates [93].

Belai studied umbilical arteriovenous PCO_2 differences in neonates with an umbilical artery pH < 7.00 in relation to neonatal encephalopathy [94]. Cut-off value of the AV-PCO_2 difference was 25 mmHg or more (sensitivity 89%, specificity 56%).

Scoring systems combining Apgar scores, or the need for resuscitation, blood gas values and fetal monitoring have claimed to be of a better predictive value towards the development of severe clinical complications, including neonatal encephalopathy [95–98]. These systems, however, have not yet been widely used.

In a group of term and preterm neonates with a pH < 7.0 a high arterial base deficit (> 16 mmol/L) and a 5-min Apgar score < 7 predicted neonatal morbidity, including cerebral complications [99].

Blood gas values cannot be used for prediction of long-term outcome. This may be caused in part by the reflection of total body metabolism with blood gases, whereas specific CNS measurements would render more specific information.

136.5.2.3 Other Biochemical Markers of Perinatal Asphyxia

Umbilical cord blood lactate levels and lactate/pyruvate ratios were found to be elevated in all full-term neonates with neonatal encephalopathy [11], and these may be more accurate than blood gases, although this has been questioned in an earlier study [87]. Cerebrospinal fluid (CSF) lactate was elevated in a small sample of asphyxiated infants within 8 hours after birth [100]. Urine lactate/creatine ratios identified newborn infants at risk for hypoxic-ischemic encephalopathy [101]. Decreased cAMP levels were described in neonates with severe neonatal encephalopathy and a poor outcome [102]. Hypoxanthine has also been used as a biochemical marker of birth asphyxia [103, 104]. Hypoxanthine correlated well with plasma malondialdehyde levels of cord blood, indicating free radical-induced oxidative stress [105]. Also non-protein bound iron and nucleated red blood cells count at birth reflect fetal hypoxia (Table 136.5) [106, 107].

All the aforementioned biochemical methods were used to determine the severity of hypoxia, but were not specific in predicting neonatal encephalopathy.

It was hoped that more specific markers of brain injury could be used for early prediction. CK-BB, the brain fraction of creatine kinase, was elevated in cord blood of term neonates with asphyxia [108]. However, the time course of CK-BB rise in peripheral blood following neonatal encephalopathy is unknown. Neuron specific enolase (NSE) in serum was significantly increased in neonates with neonatal encephalopathy, but levels of brain specific proteins (S-100B, NSE, and CK-BB) on the first day of life were of limited value in predicting a poor outcome [109, 110]. Brain specific proteins (neurofilament protein, glial fibrillary acidic protein, S-100B, NSE) in the CSF of asphyxiated neonates correlated with outcome at 1 year [111, 112].

Production of cytokines has been demonstrated in plasma and CSF and correlated with severity of encephalopathy and long-term outcome [113–118].

136.5.2.4 Clinical Scoring Systems

As described above the Sarnat score consists of a combined clinical and electrophysiological (EEG) score [25]. The Sarnat score has been used to predict long-term outcome. Whereas neonates with a score of I mostly showed a normal development and neonates with grade III were almost uniformly abnormal [31, 83, 119, 120], prediction of development in neonates with grade II neonatal encephalopathy was very difficult, as 25% had an abnormal outcome. As the Sarnat score is performed at 24 hours, its value in selection of neonates for intervention is very limited.

Wayenberg et al developed an early neurological score which was assigned at 30 min of life based on consciousness, respiratory pattern, Moro and grasp reflexes [121]. The predictive value towards the development of neonatal encephalopathy was not better than measurement of an arterial base deficit. Similar observations were done by Perlman: the triad of delivery room intubation, a five minute Apgar score of ≤ 5, an umbilical arterial pH <7.00 and/or initial arterial postnatal base deficit of ≥14 mmol/L, followed by an abnormal neurological examination at 3 ± 2.5 hours of life predicted an abnormal outcome (sensitivity 92%, specificity 52%) [122].

Thompson et al designed a clinical scoring system to quantify encephalopathy during the first few hours after birth [33]. Larger studies are needed to verify the usefulness of this system.

136.5.2.5 Neuroimaging

Cerebral Blood Flow and Oxygen Consumption

Studies in term neonates with severe neonatal encephalopathy using Doppler ultrasound or Near Infrared Spectrophotometry (NIRS) demonstrated changes in cerebral blood flow (CBF) and blood volume predicting an adverse outcome [32, 123–125]. Further studies are needed to assess the clinical value of NIRS and CBF measurements.

Cranial Ultrasound Imaging

Cranial ultrasound abnormalities, such as lesions in the thalami and basal ganglia, are usually first seen after the first day of life [126]. The presence of the abnormalities mentioned above on the first day of life, would therefore strongly suggest an antenatal onset of the insult.

Magnetic Resonance Imaging

Cranial magnetic resonance imaging (MRI) is increasingly being used during the immediate neonatal period [127–129]. MRI on the first day of life may still be normal [130]. Diffusion-weighted MRI has been extremely useful to detect early changes in hypoxic-ischemic brain tissue. By creating maps of the Apparent Diffusion Coefficient of water (ADC-maps) values of individual areas can be calculated. These ADC changes reflect cytotoxic edema, and are dependent on the time after the insult, since cytotoxic edema will change into vasogenic edema which has a different ADC value [131]. When MRI is performed before the second day of life images may still be normal since full secondary energy failure has not yet developed [132]. We and others advise to perform MRI after day 3–4 in asphyxiated full-term neonates. MRI is a good predictor of long-term motor development.

In a recent study using MRI we have demonstrated that acute insults were common in neonates with neonatal encephalopathy [4].

Different patterns of cerebral involvement after perinatal asphyxia have been described using MRI [133, 134]. In brief, abnormalities of the basal ganglia and thalamus are seen after an acute hypoxic-ischemic event such as placental abruption or uterine rupture. In the most severe cases abnormalities of the white matter can be seen. In more chronic hypoxic-ischemic events abnormalities of the white matter are encountered, sometimes in a watershed-like distribution [8]. Neonates with these abnormalities do not show any changes in basal ganglia or thalamus.

These different patterns are similar to those in animal experiments in the 1970s and in human post mortem studies [70].

Magnetic Resonance Spectroscopy

Cerebral metabolism will change during and following asphyxia. High energy phosphates will decrease, and lactate will be increased. Changes in energy metabolism can be visualized by 31-Phosphorus magnetic resonance spectroscopy (MRS). Decreased phosphocreatine and ATP levels in the first week of life correlated with an abnormal outcome [135]. In addition, the presence of a secondary energy failure could be identified in experiments and human neonates [28]. Elevated cellular pH levels could be demonstrated in neonates with perinatal asphyxia and a poor long-term outcome [136].

With 1H-MRS, increased lactate levels could be seen in brain tissue of asphyxiated neonates with a poor neurodevelopmental outcome. In addition, decreased levels of N-acetylaspartate, a neuronal marker, could be identified (Fig. 136.2) [137]. These abnormalities may persist for a prolonged period.

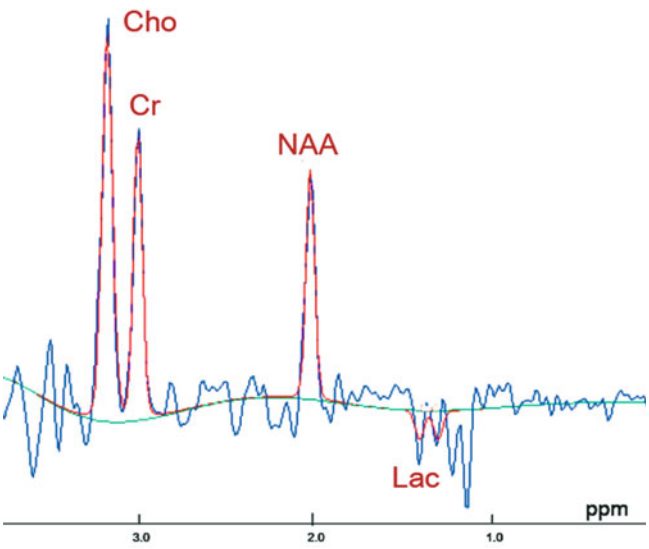

Fig. 136.2 Proton Magnetic Resonance Spectroscopy of the basal ganglia of patient A in Fig. 136.1, demonstrating a low N-acetyl-aspartate/choline ratio and elevated lactate (inverted doublet at 1.33 ppm). At 1.1 ppm the inverted doublet of propan-1,2-diol (component of phenobarbital solution) is visible (not fitted)

Neurophysiology

The electroencephalogram (EEG) has been used during the last few decades in asphyxiated neonates and is recognized to be of predictive value with regard to neurodevelopmental outcome [138–140]. These studies showed that the electrical background activity was more predictive than the presence or absence of seizure activity [138–141].

The use of conventional multichannel recordings has practical limitations. Recordings usually last 30–45 minutes, making it difficult to follow changes over a longer period of time and recognition of prolonged subclinical seizures [142]. Smaller systems have been used in neonatal intensive care units. The cerebral function monitor (CFM) was first introduced in the neonatal intensive care unit by Bjerre [143]. Since then, many studies have been performed in newborn infants using this technique [143–145]. It records a single channel EEG from biparietal electrodes. The filtered signal is rectified, smoothed and amplitude integrated before it is written out on a semilogarithmic scale at slow speed (6 cm/hr). The CFM, usually referred to as the aEEG (amplitude integrated EEG) is easy to apply and has a high concordance with the standard EEG [56, 146]. Continuous recordings can be made, allowing us to assess the background pattern and the presence or absence of sleep-wake cycling. Seizure activity can be recognized as a saw-tooth pattern. In the newer types of the devices, the raw EEG is stored on hard disk and available for evaluation. Several groups have now used the aEEG in infants with neonatal encephalopathy [143, 144]. Recovery of the aEEG background pattern within 24 hours after birth resulted in a good outcome in 61%, whereas all infants with a suppressed background pattern after 24 hours showed a poor outcome [147].

Several studies have shown that aEEG and EEG performed before 6 hours of age can be used to reliably select infants at risk of subsequent neurological handicap. Hellström-Westas et al [148] were the first to report infants, in whom aEEG registration was started before 6 hours of age. A continuous aEEG background pattern was almost a guarantee of a normal outcome. A flat or CLV pattern predicted death or a severe handicap. Very similar data were obtained by Eken et al [149]. Techniques such as aEEG are extremely useful to select neonates who may benefit from an intervention after perinatal asphyxia (Fig. 136.3) [36].

Evoked potentials (EPs) are averaged clinical responses, occurring in the EEG in response to repetitive stimuli (auditory, visual, somatosensory) and can examine the functional integrity of the sensory pathways within the nervous system in a non-invasive way [150, 151]. The EEG is of higher amplitude than the EP, but is random to the applied stimulus. The EP is of small amplitude, but constant relative to the applied stimulus and will gradually emerge from the EEG, when an adequate number of stimuli are averaged.

Both visual (VEP) and somatosensory (SEP) potentials have a very strong predictive value with regard to neurodevelopmental

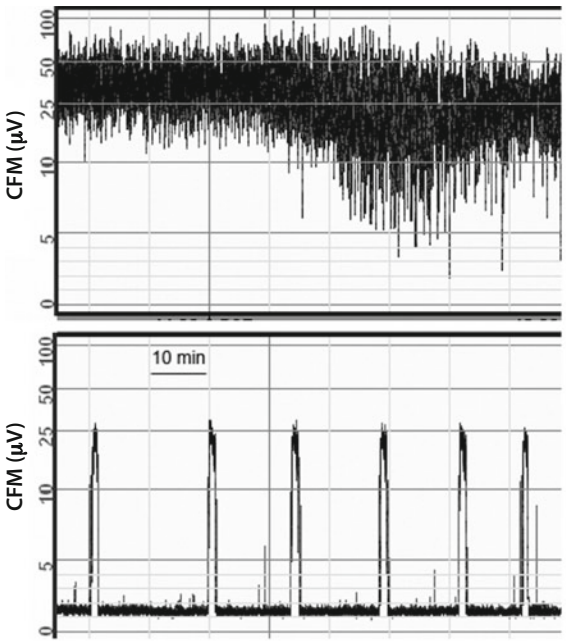

Fig. 136.3 Different aEEG patterns in asphyxiated full-term neonates written on a semilogarithmic scale (Courtesy Dr. Mona C. Toet). *Upper panel*: continuous normal voltage. *Lower panel*: repetitive seizures on a very suppressed background pattern

outcome, when performed during the first week of life [150, 152, 153]. SEPs are more likely to be abnormal on the initial test, but normalization within the first 3–4 days of life is still compatible with a normal neurological outcome [150, 153–155]. Abnormal VEPs during the first week of life were always predictive of death or cerebral palsy according to Taylor, but this was not supported by our own data.

During the last decade aEEG has replaced VEP and SEP as a bedside tool to assess the severity of neonatal encephalopathy after perinatal asphyxia.

136.6 Summary

At present, clinical observations, acidosis of cord blood, in combination with continuous aEEG monitoring are the best way to detect neonatal encephalopathy after perinatal asphyxia. Well-documented asphyxia, including need for resuscitation and neonatal encephalopathy are good predictors of a poor outcome, and could be used to select neonates for neuroprotective strategies. Patterns of brain injury can be visualized with MRI, especially diffusion-weighted MRI and ADC maps, preferably performed after the third day of life.

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137

Neuroprotective Strategies

Angela M. Kaindl, Géraldine Favrais and Pierre Gressens

137.1 Introduction

The mortality of infants, especially premature infants, has decreased remarkably in recent decades, but their morbidity has not followed the path at the same pace. Thus, neurocognitive morbidity, especially following premature birth, is a dominating health care issue. While the development of strategies to improve the neurologic outcome of infants suffering from perinatal brain damage is thereby essential, currently available intervention strategies are limited. Perinatal brain damage is believed to be a multifactorial, multihit process that varies in severity between individuals, affects infants of different genetic backgrounds and occurs at various stages of the physiological developmental program (Fig. 137.1). This said, it becomes clear that the respective pathologies, even following one type of injury, are likely multiple. Patterns of brain lesions depend on developmental stage. Periventricular leucomalacia is particularly frequent in the premature infant, while hypoxic-ischemic encephalopathy affects predominantly the term infant. The development of novel neuroprotective strategies thereby demands a clear understanding of the pathophysiology of each of the disorders in order to identify readouts or targets/pathways to test for the efficacy of candidate molecules. Since many developmental processes underlie a fine balance, careful attention needs to be paid to acute and long-term toxic effects. It remains to be elucidated, for example, when a decrease of apoptotic cell death is beneficial or detrimental.

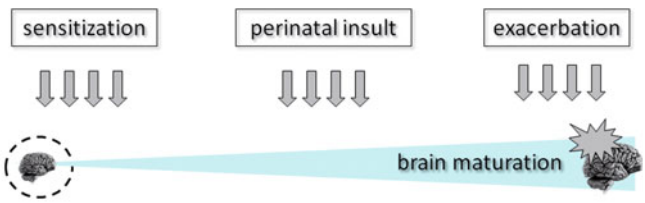

Fig. 137.1 Multiple-hit hypothesis for the development of perinatal brain damage. Schematic representation illustrates the multiple-hit hypothesis including pre-, peri- and postnatal factors. Several risk factors are implicated and are frequently associated with the pathogenesis of perinatal brain damage in animal models. These comprise prenatal risk factors such as inflammation/cytokine release and maternal stress, perinatal factors such as hypoxic-ischemic stimuli, and postnatal factors such as growth factor deprivation, inflammation/cytokine release, drug side effects and pain (see Table 137.1). Furthermore, a combination of such factors in experimental models has resulted in the emergence of a multiple-hit hypothesis, which consists of a sensitization state created by a mild first event, leading to an increased susceptibility to a second injury. These factors may cause various lesions depending also on the developmental stage of the brain

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137.2 Pathophysiology of Brain Diseases and Definition of Targets

The development of neuroprotective strategies is based on fundamental knowledge of the induction and progression of brain damage. For this, brain pathology has been studied in postmortem tissues from patients as well as in animal models that mimic human pathology. Such approaches have allowed various intertwined causes and mechanisms that induce white and/or grey matter damage of the central nervous system to be distinguished (Table 137.1), for review see [1]).

Table 137.1 Risk factors for encephalopathy of prematurity

Antenatal factors	Perinatal factors	Postnatal factors
Inflammation	Hypoxia-ischemia	Oxidative stress
Hypoxia-ischemia	Excitotoxicity	Inflammation
Toxins	Oxidative stress	Pain
Malnutrition	Loss of maternal GF	Excitotoxicity
Maternal stress	Drugs	Drugs
Genetic factors	Genetic factors	Loss of maternal GF Genetic factors

GF growth factor.

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Owing to epidemiologic and experimental studies, neonatal brain susceptibility to perinatal insults is well known. Periventricular white matter damage (PWMD) affects preterm neonates born between 23 and 32 weeks of gestation and follows successive pathologic events from pre- to postnatal period. In parallel, brain lesions proceeding from acute hypoxia-ischemia in at term neonates involve an apoptotic/necrotic process leading to early and delayed neuronal cell death in the gray matter, mainly at thalamic level. Pathophysiologic events in conditions of perinatal brain damage that represent targets for neuroprotective therapy are summarized in Table 137.2 (Figs. 137.2 and 137.3).

Table 137.2 Pathophysiologic events in conditions of perinatal brain damage that represent targets for neuroprotective therapy

Primary and secondary energy failure	– In situations of reduced oxygen supply the metabolism is redirected towards anaerobic glycolysis to reduce the requirement for oxygen – This leads to a decreased production of ATP and primary energy metabolites and thus to a secondary energy failure
Excitotoxicity	– Energetic breakdown induces a surge of glutamate that accumulates in the synaptic gap due to cell damage and the inability of astrocytes to recycle these high levels of the neurotransmitter at an adequate pace – Excitotoxicity refers to the deleterious effects of such high glutamate levels on brain tissue: overactivation of glutamate receptors, massive influx of calcium, secondary cell damage, etc
Oxidative stress	– Oxidative stress, i.e., an increase of free radicals, results from activation of multiple pathways such as excitotoxicity or microglia activation – This can lead to fragmentation of DNA and of membranes and thereby trigger necrotic cell death – During the fetal and neonatal phase, recycling of such free radicals by scavengers is limited, and this renders the developing brain particularly vulnerable to oxidative stress
Mitochondrial failure and cell death	– Mitochondria are central organelles implicated in physiological energy supply, but also in the process of cell death following initial brain injury through activation of the cytochrome C and caspase pathways
Microglia activation	– Microglia, the resident macrophages of the central nervous system (CNS), constantly screen the CNS and can be activated rapidly through various environmental changes. In the activation process, microglia change their phenotype, proliferate, migrate to the site of damage and secrete pro-inflammatory factors and participate in the generation of oxidative stress (of note, a protective role of microglia is also known)
Increase in brain blood barrier (BBB) permeability	– Brain damage increases the permeability of the BBB and thereby facilitates the infiltration of immune cells (e.g., macrophages, lymphocytes), toxic molecules and of water from the circulating blood pool – One reason for the dysfunction of the BBB is the degradation of tight junctions within this barrier by increased activation of matrix metalloproteases (MMPs) in the extracellular matrix
Neuroplasticity	– Neuroplasticity refers to changes that occur in the organization of the CNS throughout brain development and also within the mature brain – Following lesions, various phenomena of plasticity and reparation can appear such as increased rates of stem cell proliferation and differentiation

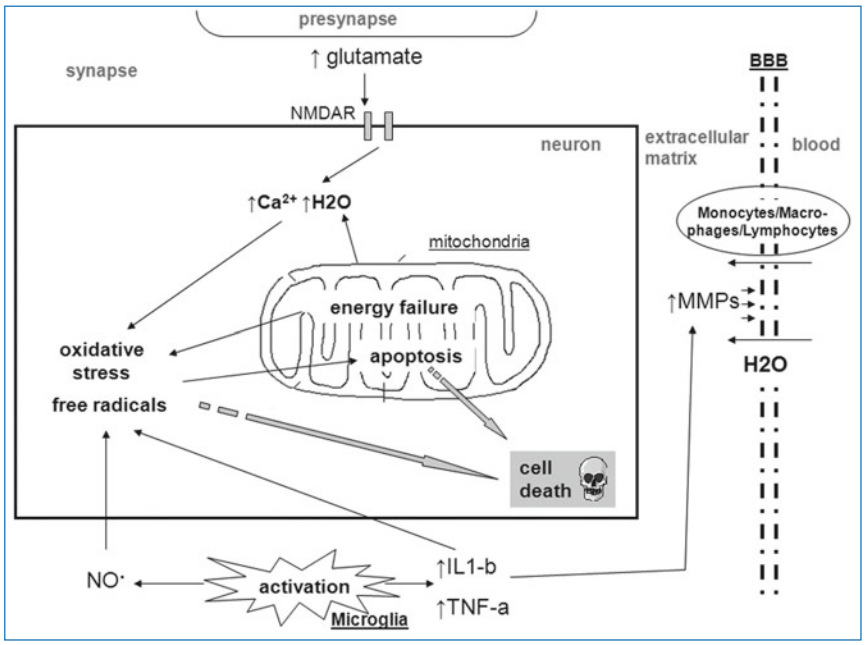

Fig. 137.2 Pathophysiologic mechanisms implicated in cell death following a brain lesion. *MMPs* metalloproteinases, *NO* nitric oxide, *NMDAR* N-methyl-D-aspartate receptor, *BBB* blood brain barrier

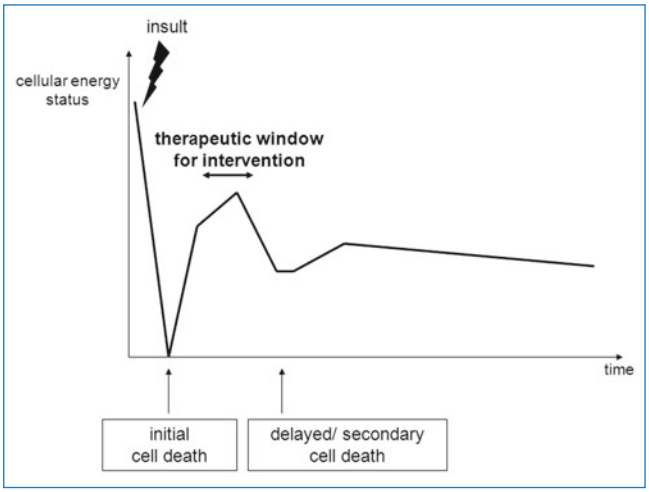

Fig. 137.3 Kinetics of cellular energy status following an acute brain lesion. Results of an *in vivo* study of phosphate metabolites during hypoxic-ischemic brain damage have permitted a biphasic kinetic of the energy breakdown to be identified. Primary energy breakdown occurs in the initial phase of brain damage. Later, during reperfusion, a transitory reconstitution of ATP and antioxidant levels is noted, preceding a further dip in the production of ATP. The latter is referred to as secondary energy breakdown and occurs about 4–8 hours after the initial damage. Its time of occurrence offers a feasible therapeutic window for neuroprotective strategies. Modified from [2]

137.3 Neuroprotective Strategies: Experimental Results

Deciphering the nature and temporal appearance of phenomena that follow damage to the cerebral parenchyma allows for the identification of potential therapeutic targets and the therapeutic window preceding the onset of secondary cell death. Various therapeutic strategies have been tested *in vitro* and *in vivo* in animal models. Principal limiting factors of these pharmacologic approaches are 1) the passage of molecules via the BBB, 2) the potential toxicity of applied molecules, 3) the therapeutic window and thus the existence of a beneficial effect even when a molecule is applied relatively late after a brain damage, and 4) the persistence of a long-term protective effect.

Several molecules have been shown to be efficient in the reduction of lesion sizes/extents and their consequences. In general, neuroprotective approaches can include mechanisms such as an inhibition of sensitizing factors, preconditioning against damage, a pharmacological blockage of mechanisms involved in primary or secondary damage or treatment that increases endogenous reparation processes or cause reparation by themselves (Fig. 137.4). Even though these drugs usually act on several levels of a damage cascade, we will classify them below according to their most prominent (or most studied) effect.

137.3.1 Anti-inflammatory Agents

137.3.1.1 Minocyclin

Minocyclin is an antibiotic of the tetracycline family and can pass the blood-brain barrier (BBB). In addition to its antibacterial effect, its administration immediately after a hypoxic-ischemic or infectious damage to the brain in animals has disclosed a significant reduction of microglia activation, of pro-inflammatory cytokine production and of oxidative stress induction in the brain. This anti-inflammatory effect is accompanied by a reduction of matrix metalloproteinase (MMP) production and an increased tightness of the BBB by magnetic resonance imaging. Minocyclin also has anti-apoptic properties that are mediated on the level of mitochondrial transition permeability. This pluripotent molecule is well tolerated by adults, and a positive effect in the treatment of neurodegenerative diseases has been demonstrated. However, the effect of minocyclin regarding an improvement of the neurologic prognosis stays controversial. In addition, minocyclin is contraindicated in children below an age of 9 years.

137.3.1.2 Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

Cyclo-oxygenases are essential for the formation of prostaglandins and thromboxan from membranous arachidonic acid. Two types of cyclo-oxygenase are distinguished: the cyclooxygenase type 1 (COX-1) or constitutive type and the cyclooxygenase type 2 (COX-2) or inflammation inducible type. NSAIDs that selectively block COX-2, such as nimesulid, are preferably applied, even though positive results have been obtained with indomethacin and ibuprofen. Nimesulid has been shown to have a neuroprotective effect in models of focal and diffuse ischemia and systemic inflammation

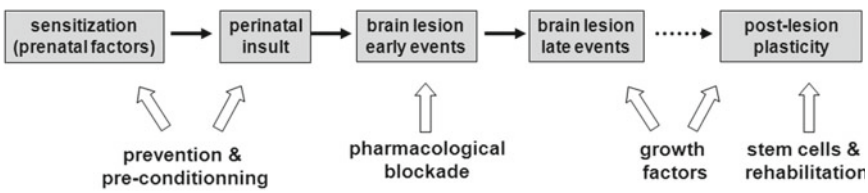

Fig. 137.4 Schematic representation of the potential strategies for neuroprotection against perinatal brain damage

in a sustained manner despite a late application up to 6 hours after the acute incident. Neuroprotective mechanisms include an inhibition of TNFa expression while increasing that of interleukin-10 (IL-10), a prevention of BBB dysfunction and a reduction of oxidative stress [3–5]. These promising results have not been confirmed in humans so far. Thus, their administration needs to be seen with caution in the context of inflammatory brain diseases, also because of potentially toxic effects in the newborn in which the hepatic metabolism for NSAIDs is reduced.

137.3.2 Excitotoxicity Reducing Agents

Strategies developed to limit excitotoxicity are based on an inhibition of postsynaptic glutamate receptors. Thereby, the principal difficulty is to block toxic/overstimulated receptors without inhibiting the physiologic function of these receptors essential for normal brain development and function. It has been demonstrated that an unselective inhibition of the N-methyl-D-aspartic acid (NMDA) receptor in critical periods of brain development triggers apoptosis, inhibits cell proliferation and causes neurobehavioral deficits in rodents. Still, blockage of NMDA receptors, especially of certain subunits, can be neuroprotectrive, as has been shown in various animal models for example using memantine. This paradox illustrates the difficulty in the balance between beneficial and harmful effects of neuroprotective strategies in the course of brain development.

137.3.2.1 Magnesium

Magnesium binds NMDA receptors and inhibits their activation; this block is removed by membrane depolarization. Thereby, magnesium possesses a modulatory role and inhibits excitotoxic stress. An anti-apoptotic effect has been demonstrated in animal models. The administration of magnesium to pregnant women presenting with threatening premature birth has shown to be beneficial with regard to the incidence of cerebral palsy and motor dysfunction in the infant born prematurely [6] with a good tolerance of the protocols administrated [7]. In adult victims of traumatic brain injury, epidemiologic studies have identified low magnesium levels < 1.3 mmol/L as a negative prognostic factor [8]. Still, a prolonged intravenous supplementation of high doses of magnesium was not beneficial, and the passage of magnesium across the BBB in the adult is uncertain [9, 10].

137.3.2.2 Topiramate

The antagonism of α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and kainat receptors, for example through topiramate, has not shown effects as deleterious on neuronal survival as those acknowledged for NMDA receptor antagonists in the developing brain. In addition, a protective effect of topiramate on preoligendrocytes, cells that have been shown to be severely affected after an excitotoxic stress or hypoxic-ischemic stress in the neonatal period, has been demonstrated [11]. Topiramate has been approved for use as an antiepileptic drug in children older than 2 years. So far, topiramate is not used as a neuroprotective agent but its good tolerance and further experimental data argument for further analysis of this application.

137.3.3 Antioxidants

Two approaches have been developed to limit oxidative stress. The first consists of a reduction of free radical production through xanthine oxidase inhibition, of lipid peroxidation and of inducible nitric oxide synthase (iNOS). The second approach consists of increasing antioxidant defense mechanisms through an increase of reactive oxygen species (ROS) and reactive nitrogen species (RNS) scavengers.

137.3.3.1 Allopurinol

Allopurinol is an inhibitor of the xanthine oxidase. Its neuroprotective action, particularly through the reduction of oxidative stress and of cerebral edema, and its good tolerance has been shown in various animal models; this supports the realization of clinical studies in humans. It has been employed in therapeutic essays in infants with hypoxic-ishemic encephalopathy. In a first study, a reduction of the nitric oxide (NO) concentrations at 72–96 hours of life were measured in the serum of newborns suffering of moderate to severe hypoxic-ishemic encephalopathy following allopurinol treatment. This was associated with an amelioration of the neurologic outcome at 12 months of life [12]. Despite this, a meta-analysis of various therapeutic essays realized for this indication has not been able to show a beneficial effect of allopurinol on mortality or on progression of neonatal convulsions [13].

137.3.3.2 Vitamin E

Vitamin E has an anti-oxidant effect through the elimination of free radicals. On the other hand, high doses have been shown to induce neuronal apoptosis *in vitro* [14]. In newborns, vitamin E supplementation reduces the risk of intraventricular hemorrhage and of retinopathy but favors the progression of sepsis, especially if its serum concentration is above 3.5 mg/dL [15]. This toxic effect of high concentrations demands caution when using this agent as a neuroprotector.

137.3.3.3 N-acetylcysteine

N-acetylcysteine (NAC) induces the production of glutathione which permits the clearance of free radicals. In

various animal models, especially those of maternal-fetal infection, a robust neuroprotective effect has been found which is accompanied by a reduction of oxidative stress, of pro-inflammatory cytokine production and of apoptosis [16, 17]. No secondary effects were observed when NAC was administered to humans in the framework of clinical studies. In the premature infant, systemic parenteral nutritional supplementation neither modified the mean mortality at 36 weeks of gestation, nor the frequency of retinopathy, periventricular leucomalacia or intraventricular hemorrhage [18]. A randomized controlled clinical study is currently being performed in children.

137.3.3.4 Melatonin

Melatonin is a hormone that participates in sleep regulation and that is secreted physiologically by the pineal gland. Its role as a neuroprotector has been shown in several animal models in the framework of degenerative diseases such as acute brain lesions. Two of the implicated mechanisms seem to dominate this effect. First, its antioxidant effect can inhibit the NO synthase and lipid peroxidation as well as RNA degradation, favoring the transcription of other antioxidant enzymes. Further, in acute lesion animal models, melatonin favors axonal outgrowth with a long-term improvement of neurocognitive function in comparison to the control animals [19–21]. Melatonin is well tolerated and crosses the BBB passively. In the near future, this drug should be used in clinical studies in order to evaluate it efficacy as a neuroprotective agent in humans.

137.3.4 Prevention of Protracted Cell Death and Modulation of Plasticity

Possible strategies against apoptotic cell death or those that favor mechanisms of reparation are particularly interesting because the treatment window relative to the initial damage is particularly large.

137.3.4.1 Erythropoietin

Erythropoietin (Epo) is a hematopoietic growth factor whose secretion is sensible to hypoxia. It is also a ubiquitous cytokine for which receptors exist on cells of the central nervous system. Moreover, it is currently applied in its recombinant form (rEpo) in neonatal medicine for treatment of anemia of prematurity and because of that has also been tested in experimental animal models regarding its effect on lesions of the developing brain. Experimental data have demonstrated an anti-apoptotic effect. This effect is accompanied by a reduction of pro-inflammatory cytokines, a reduction of oxidative stress, a recuperation of the energetic level and an increase of trophic factors involved in reparative processes [22–25]. In animal models, often high doses of rEpo are applied (higher than those currently used in the human newborns), and the beneficial effects are dose-dependant [25]. Using the currently applied doses, no study has been able to show a beneficial effect on brain development [26]. Due to concern regarding negative effects such as thrombosis and hypertension in adults with kidney failure, clinical trials have been placed on hold.

137.3.4.2 Progesterone

The better outcomes of female subjects in behavioral tests after a cerebral lesion have drawn attention to the neuroprotective potential of sex hormones, in particular of progesterone. In fact, treatment of animals with intravenous progesterone has shown a neuroprotective effect in various animal models, particularly in adult ovariectomized subjects. In parallel, progesterone is also secreted by glial cells independent of the sex, especially in the inflammatory context. The neuroprotective and neurotrophic effect of progesterone is multifactorial. Its modulatory effect on postsynaptic receptors, particularly on $GABA_A$ receptors, and on transmembrane ion movements via Na-K-ATPases has been most extensively studied. It also has an anti-edematous effect. Progesterone has been the focus of several clinical phase II studies in human adults particularly in the treatment of traumatic brain injury (TBI) where a good tolerance, a reduction of mortality and an amelioration of the neurologic outcome 6 months after TBI has been shown. So far, no study has been performed in children.

137.3.4.3 Cyclosporine A

Cyclosporine A is an immunosuppressant and has been shown to be neuroprotective in various models of brain damage including TBI and hypoxia-ischemia in adults. This is in part due to its ability to inhibit an increase of mitochondrial permeability, an initial step in apoptotic cell death preceding the breakdown of mitochondria, and to modulate calcineurin which intervenes in the regulation of ion channels and the synaptic plasticity favoring axonal regrowth. A phase II clinical study has established the doses permitting a good tolerance after a TBI in adults prior to tests regarding its efficacy on the neurologic outcome.

137.3.4.4 Hypothermia

Since the early 90s, hypothermia has been in the focus of several animal studies regarding its neuroprotective effect following hypoxia-ischemia or traumatic brain injury. Results

of these studies have indicated that hypothermia reduces secondary energy breakdown, oxidative stress, BBB rupture extent and apoptotic cell death (for review see [27]). These studies have allowed the window for therapy initiation (within 6 hours of life), the duration of therapy (72 hours) and the ideal cooling temperatures (33–35°C) to be determined. The clinical studies have allowed the relative innocuousness of moderate hypothermia in the term newborn to be established and have only shown individual cases of sinus bradycardia and thrombopenia without hemorrhage. Two cooling procedures have been applied in these clinical studies with the indication of hypoxic-ischemic encephalopathy stage II or III: 1) selective head cooling using a cooling cap with a maintenance of the body temperature at 34.5°C and a gradient between the scalp (25°C) and the deep brain structures (35°C); 2) a cooling of the entire body to 33.5°C leading to a homogenous cooling of the cerebral structures. So far, clinical studies have not allowed a difference between the two currently available protocols to be convincingly demonstrated. Recent experimental results demonstrated different susceptibilities of various brain structures towards hypothermia. The results of clinical studies (two were randomized controlled multicenter studies) in the newborn suffering from a stage II or III hypoxic-ischemic encephalopathy have been reviewed in a metaanalysis published in 2007. The latter supported the notion that moderate hypothermia has a benefit on mean mortality and severe handicap at 18 months of age [28]. Currently, another multicenter study (TOBY) is being performed and appears to support previous positive results. Moreover, several studies now focus on the combination of hypothermia with other agents such as rEPO.

137.3.4.5 Xenon

Xenon is an odorless gas that exists in the Earth's atmosphere in traces, can be produced by distillation processes from air and is currently being evaluated regarding is neuroprotective potential with regard to perinatal brain damage.

137.3.5 Stem Cell Therapy

The development of an adequate protocol for stem cell culturing and application has permitted the use of these cells for the reparation of cerebral lesions to be envisaged. While some studies have shown a positive effect of stem cell therapy on the lesion size/extent and/or outcome following brain lesions, it is not clear yet whether the stem cells themselves or factors secreted by stem cells mediate the positive effect. In case stem cell integration is important for their effect, these cells need to proliferate, find the site of lesion and differentiate into an adequate cell type (e.g., neuron, oligodendrocyte) and integrate into the tissue to be functional. The ethical problem associated with the use of human stem cells is less evident in mesenchymal stem cells that can be taken from cord blood or when induced pluripotent stem cells (iPS) are used. Such cells permit an autologous transplant and do not entail the problem of immune tolerance of the transplanted cells. A clinical study is currently being performed using stem cells in children with neurodegenerative diseases at the Duke University.

A further intriguing alternative to treatment with stem cells is to stimulate the production of endogenous neuronal stem cells. It has already been shown that stem cells accumulate in the subventricular zone following an acute brain lesion. These results open a new perspective: the stimulation of this stem cell population to support the physiological reparation processes of a lesion. Still, these cells will need to integrate and function correctly in the damaged tissue (for review, see [29]). Moreover, stimulation of stem cell proliferation bears the risk of cancer induction.

137.4 Neuroprotective Strategies and the Reality

Only a few drugs that have been shown to be neuroprotective in experimental models of perinatal brain damage are being studied in clinical randomized controlled studies. There are several reasons for this, including the hesitation to carry out and finance studies in infants and the uncertainty of innocuousness on development. Moreover, the neuroprotective effect of numerous molecules has been confirmed only in clinical studies performed in adults [30]. Therefore, despite an increase in research initiatives in this field, neuroprotective measures are limited in everyday clinical practice. Moderate hypothermia has been shown to improve the neurologic outcome of infants with stage II and III hypoxic ischemic encephalopathy. Despite all the positive results, hypothermia still needs to be applied with caution and in a controlled fashion since the long-term outcome of treated infants has not been sufficiently studied and so far unknown complications may occur. A central registry permitting evaluation of the clinical practice of hypothermia is warranted. In parallel, the treatment of magnesium in mothers in the case of imminent premature birth has been shown to be positive with regard to the outcome of premature infants. Other neuroprotective strategies consist mainly of preventing aggravating situations such as pain, cardiovascular instability and hyperthermia.

137.5 Conclusions

The neuroprotective concept and protocols for neuroprotective strategies have been largely developed through experimental studies. Despite this, the application in humans is limited due to difficulties of performing clinical studies in

infants with drugs that have for the most part not been FDA approved at that age in their primary indication, due to the heterogeneity of the population and the mostly moderate results in animal models. Moreover, a world-wide systematization of clinical studies on neuroprotective strategies is needed. Since the pathologies are not homogeneous, several molecules or techniques may need to be combined to further ameliorate the neurologic prognosis.

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Cerebral Hemorrhage

Linda S. de Vries

138.1 Neonatal Intracranial Hemorrhage in Preterm Infants

In the late 1970s, computed tomography (CT), and shortly afterwards cranial ultrasound (cUS), was used for the first time to visualize intracranial lesions [1]. cUS initially used the temporal bone but soon the anterior fontanelle was preferred as an acoustic window. The first cUS studies used a low resolution linear mechanical sector transducer, limiting the field of view to the lateral ventricles. The adjacent white matter was poorly visualized, until mechanical sector scanning was introduced with a wider angle of insonation. Data collected in the early years is therefore very different from the data that can be obtained today, using high resolution cUS as well as magnetic resonance imaging (MRI). Since the more routine use of neonatal MRI, it has become clear that injury of the vulnerable white matter of the preterm infant is more important than hemorrhages in the germinal matrix and the ventricles (germinal matrix hemorrhage-intraventricular hemorrhage [GMH-IVH]). Lesions that were in the past visualized as GMH-IVH are often associated with subtle injury to the white matter, which can either be entirely overlooked using cUS or underestimated. Long-term outcome data of preterm infants studied in the eighties with cUS therefore need to be interpreted with care. A decline in the incidence of severe white matter lesions, referred to as cystic periventricular leukomalacia (PVL) has been reported [2, 3]. However, although a decline in the overall incidence of GMH-IVH has been reported as well, there appears to have been no decline in the incidence of more severe grades of GMH-IVH and these severe hemorrhagic lesions still have a major impact on neurodevelopmental outcome [2, 4]. Use of additional acoustic windows to the anterior fontanelle, for example the mastoid window, has allowed for the recognition of hemorrhagic lesions of the cerebellum, especially in very immature and extremely low-birth weight infants [5, 6].

138.2 Neuropathology and Pathogenesis

The germinal matrix area is highly vascularized and is described as an immature vascular rete as the vessels within the germinal matrix are primitive and cannot be classified as arterioles, venules or capillaries. It is a transient structure, initially the site of neuroblast and glioblast mitotic activity, before cells migrate to other parts of the brain. Once cell division and migration is complete, the germinal matrix progressively decreases in size with regression being almost complete by term equivalent age. The germinal matrix area is most abundant over the head and body of the caudate nucleus, but is also seen in the roof of the temporal horn until approximately 33–34 weeks of gestation. MRI has recently confirmed the abundance of the germinal matrix and has shown that small GMHs, which were not identified on cUS, may often be seen on MRI in the temporal horn. A large autopsy series from New Jersey reported that more than a third of cases with germinal matrix hemorrhage had involvement of the temporal or occipital germinal matrix [7]. The arterial supply of the germinal matrix is from the recurrent artery of Heubner (a branch of the anterior cerebral artery), as well as terminal branches of the lateral striate arteries. Venous drainage of the deep white matter occurs through a fan-shaped leash of short and long medullary veins through which blood flows into the germinal matrix and subsequently into the terminal vein which lies below the germinal matrix [8]. This has led to the understanding that a unilateral parenchymal hemorrhage is due to venous infarction [9, 10] or to reperfusion injury following an ischemic insult.

It is most often considered that hemorrhage arises from the thin walled veins [11, 12], although Pape and Wigglesworth [13] suggested from injection studies that capillary bleeding was more common than terminal vein rupture. Ment et al [14]

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

have suggested that germinal matrix vessels change significantly over the first days of life to develop greater continuity of the basement membrane. This rapid maturation, presumably as a result of early birth, may be one of the reasons why GMH-IVH usually occurs during the first few days of life.

138.3 Risk Factors Related to the Prenatal and Perinatal Periods

In the prenatal period, amniotic infection, diagnosed by histology, has been shown to increase the risk of GMH-IVH [15]. A correlation was shown between raised blood cytokine concentrations and altered hemodynamic function [16]. In a recent study, the presence of clinical maternal chorioamnionitis was associated with an increased risk of early sepsis (OR, 5.5; 95% [CI] 2.9–10.7) and severe intraventricular haemorrhage (OR, 1.6; 95% [CI] 1.2–2.2), adjusted by multivariate regression analysis for illness severity [17].

There is no evidence that cesarean section protects the premature infant against GMH-IVH. In a recent hospital based study of preterm infants with a birthweight of ≤ 1250 g, elective cesarean section had no beneficial effect on either mortality or neuro-disability at two years of age [18]. Breech delivery is in some studies associated with a higher risk of large GMH-IVH, but this effect was lost in multivariate analysis [19]. In another study, cesarean section was beneficial for the most immature infants with a gestation < 27 weeks [15]; reduced mortality, but not a reduced risk of a GMH-IVH, was reported in a Swedish population-based study of preterm infants with a gestation of 25–36 weeks [19].

Delayed cord clamping has been demonstrated to reduce GMH-IVH with five [14%] in the delayed clamping group compared with 13 [36%] in the non-delayed group; $P = 0.03$) [20]. When the impact of delayed cord-clamping on IVH was evaluated adjusting for gestational age and cesarean section, the final model indicated that the IVH rate was > 3 times higher in the immediate cord clamping group ([OR]: 3.5, 95% [CI] 1.1–11.1). In a systematic review by Rabe et al, no significant differences for infant deaths was found, but a significantly increased incidence of IVH was reported in seven of the ten published studies (p = 0.002) [21].

Infants born outside a perinatal center and transported to one also have a higher incidence of GMH-IVH [15, 22, 23]. The NEOPAIN trial showed that outborn babies were more likely to have severe IVH (p = 0.0005) and this increased risk persisted after controlling for severity of illness, but when adjustments were made for use of antenatal steroids, the effect of birth center was no longer significant [23].

Cardiovascular and respiratory problems have always been considered to play a major role in the development of a GMH-IVH in the immediate neonatal period. Fluctuations of the intravascular pressure or blood flow may lead to rupture of the immature vascular rete in the germinal matrix and hemorrhage is known to occur during reperfusion following a period of hypotension. Studies of flow in the superior vena cava during the first hours after birth, showed that low flow preceded a GMH-IVH [24]. Although the possible lack of cerebral autoregulation, rendering the cerebral circulation pressure-passive is still considered important, it is not present in all preterm infants [25]. Continuous monitoring with near infrared spectroscopy may be a useful tool in the identification of infants at risk [26]. Respiratory risk factors can occur with complications during mechanical ventilation, such as vasodilatation secondary to hypercapnia, for instance following a pneumothorax [27]. Improvement in ventilatory techniques, increased use of non-ventilatory support, such as CPAP and even variable/bilevel positive airway pressure BIPAP are likely to further reduce the incidence of GMH-IVH.

138.4 Genetic Factors

More recently there has been an interest in the role of thrombophilic disorders in the development of GMH-IVH. Factors include heterozygosity for factor V Leiden mutation, which renders factor V resistant to cleavage by activated protein C, and heterozygosity for prothrombin G20210A mutation, which was found to be associated with raised plasma concentrations of prothrombin. A large prospective study was unable to confirm previously reported associations between the above mentioned gene variants and development of intraventricular hemorrhage in very low birth weight infants [28]. Interleukin-6 CC genotype increased the risk of the development of severe hemorrhagic lesions ([OR]: 3.5; 95% [CI]: 1.0–12.2; $P = 0.038$) [29]. However, this observation could not be confirmed in a considerably larger sample [30]. Antenatal porencephaly in two preterm siblings was recently reported in association with a mutation in the collagen 4 A 1 gene (*COL4A1*) encoding procollagen type 4 α1, a basement membrane protein [31].

A reduced risk of GMH-IVH was found with maternal pre-eclampsia. This appears to be due to enhanced in-utero maturation of the fetus, associated with a reduced risk of postnatal development of respiratory distress [32].

Routine use of inhaled nitric oxide (iNO) in intubated preterm infants seems to show a reduction in the risk of severe IVH or PVL (typical RR 0.70 [95% CI 0.53, 0.91]; typical RD - 0.07 [95% CI - 0.12, - 0.02]). However, early rescue treatment with iNO was associated with a trend towards increased risk of severe IVH [33].

138.5 Incidence of GMH-IVH

The incidence of GMH-IVH is directly related to the maturity of the infant [15]. Initial studies in the late 1970s and early 1980s showed an incidence as high as 40–50% in those weighing

< 1500 g [1, 34] but these numbers reduced to 20% in the 1990s according to some studies [4, 22] but not others [35]. The average incidence of a unilateral parenchymal hemorrhage varies from 3–11% with the lowest incidence in a French population based study [36–38]. No decrease in this type of lesion was seen by Hamrick et al [2] in contrast to their decrease in cystic white matter disease and in a recent study by Sarkar [39].

138.6 Diagnosis

Even though MRI is increasingly being used, GMH-IVH is still more likely to be diagnosed during routine bedside cUS. Performing ultrasound as part of the admission procedure and several times during the first week, allows accurate timing and identification of lesions of antenatal onset. Almost all hemorrhages will have developed by the end of the first week after birth and many within the first hours after birth. Only about 10% of the GMH-IVH occur after the first week. This is in contrast to white matter injury, where a late onset is not uncommon [40]. Progression from small GMH-IVH to a parenchymal hemorrhage can occur and is most likely related to impaired venous drainage of the medullary veins in the white matter with obstruction at the site of the germinal matrix [36].

Table 138.1 Classification of cerebral hemorrhage

Description	Generic term
Grade I: Germinal matrix hemorrhage	GMH-IVH
Grade II: Intraventricular hemorrhage without ventricular dilatation	GMH-IVH
Grade III: Intraventricular hemorrhage with acute ventricular dilatation (clot fills > 50% of the ventricle)	GMH-IVH and ventriculomegaly
Hemorrhagic parenchymal infarction (describe size, location)	HPI

Modified from [41].

Clinical diagnosis does not play a major role, as most cases of the GMH-IVH will be silent. Three clinical syndromes have been described [41]. The first is associated with a catastrophic deterioration, with a sudden deterioration in the clinical state of the infant, such as a sudden fall in blood pressure and or metabolic acidosis. It is however more common to find a sudden fall in hemoglobin without a clear change in the condition of the child. The second clinical syndrome is a saltatory one, with a more gradual onset, presenting with a change in general movements. The silent, asymptomatic syndrome is most common and can even occur in infants who show a parenchymal hemorrhage on a routine repeat ultrasound examination.

Volpe suggested a classification system [41] to describe early and late ultrasound appearances (Table 138.1). The use of grade IV is avoided and a separate description of the size, site and appearance of a parenchymal lesion is preferred. It is not always possible to distinguish between a small hemorrhage restricted to the germinal matrix and a GMH with some blood ruptured through the ependyma into the ventricular lumen. The use of the posterior fontanelle as an alternative acoustic window has been advocated by Correa et al [42] showing improvement in the diagnosis of a small IVH (Fig. 138.1). GMH at sites other than the head of the caudate nucleus, like the roof of the temporal horn, often remain undiagnosed and will only be diagnosed when an MRI is also performed [43].

A large IVH can be confidently identified by US, although associated white matter damage may be more reliably diagnosed with early MRI (Figs. 138.2, 138.3). Distinguishing a large, bulky choroid plexus, which is common in very immature infants, from a large IVH is not always easy and sequential examinations and examination through the posterior fontanelle may help. Blood can acutely dilate the ventricle or can lead to posthemorrhagic ventricular dilatation (PHVD) a few weeks later; the larger the amount of blood, the more likely for this to occur. Blood spreads rapidly through the foramen of Monro into the third ventricle, the aqueduct of Sylvius, the fourth ventricle, the foramina of Magendie and Luschka and eventually into the posterior fossa. Clot formation can

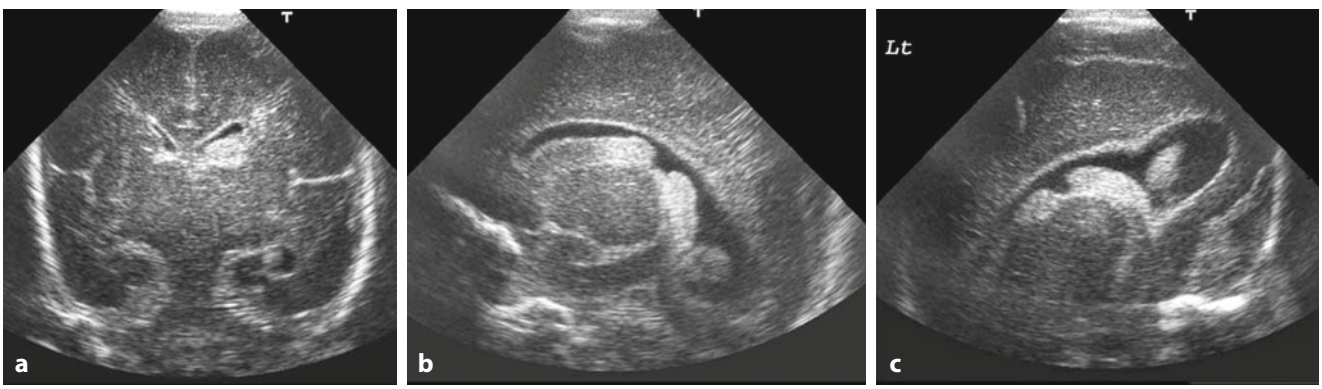

Fig. 138.1 Cranial ultrasound of a preterm infant born at 29 weeks GA, coronal and parasagittal views, taken through the anterior (**a**, **b**) and posterior (**c**) fontanelle, showing an intraventricular clot and a dilated occipital horn

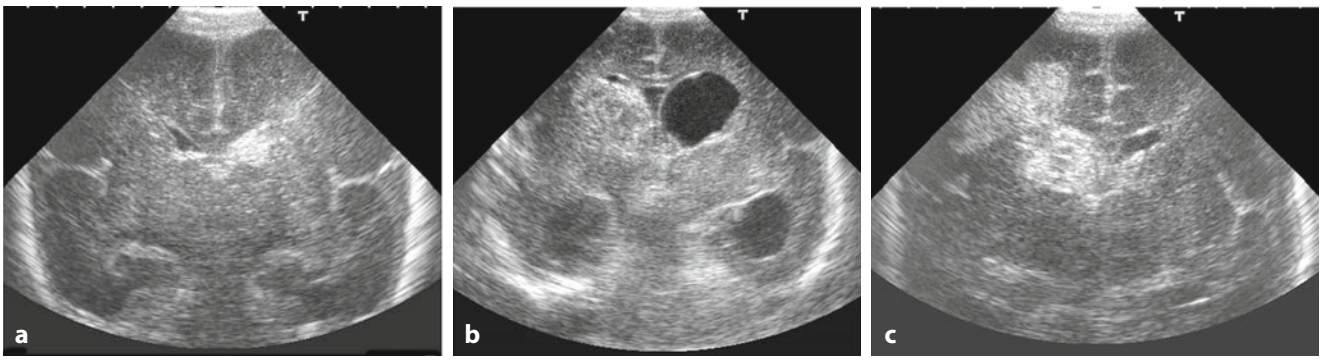

Fig. 138.2 Cranial ultrasound, coronal views, of three preterm infants, showing a small IVH with focal ipsilateral periventricular echogenicity (**a**), a large bilateral intraventricular hemorrhage with acute ventricular dilatation (**b**) and a parenchymal hemorrhage and large IVH (**c**)

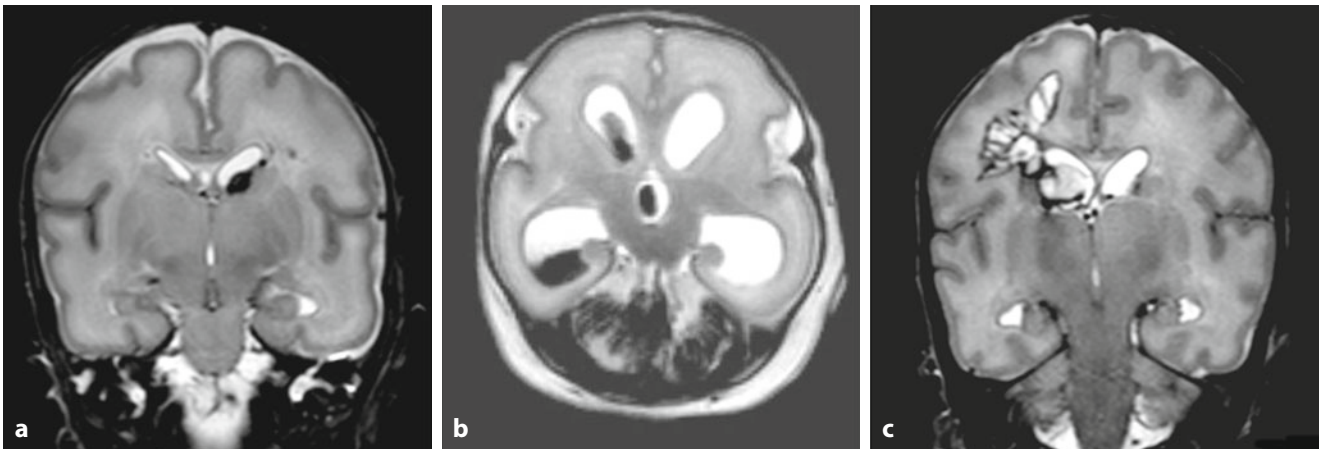

Fig. 138.3 MRI (T2 weighted sequence) of the three preterm infants shown in Fig. 138.2; IVH associated with petechial hemorrhage in the left periventricular white matter (**a**); besides large IVH, bilateral cerebellar hemorrhages are seen (**b**); IVH and PHI, similar to cUS (**c**)

occur at any level and lead to outflow obstruction, but it is most commonly seen at the level of the aqueduct or, more diffusely, in the posterior fossa. Depending on the degree of blood and the site of obstruction, PHVD can be rapidly progressive and non-communicating; it is usually due to obstruction at the level of the aqueduct of Sylvius, or, if more gradual in onset and communicating, due to obliterative arachnoiditis.

A unilateral parenchymal hemorrhage, referred to in the literature as hemorrhagic parenchymal infarction (HPI) or venous infarction (VI) accounts for 3–15% of all GMH-IVH [36–38]. It is usually unilateral, triangular in shape, with the apex at the outer border of the lateral ventricle and associated with a moderate to large ipsilateral GMH-IVH. It has been thought in the past that parenchymal hemorrhage was due to direct extension of hemorrhage into the periventricular white matter, but this is no longer considered to be the most likely explanation for this type of parenchymal lesion. Most would now agree, that this type of lesion is caused by the presence of GMH-IVH, which can lead to impaired venous drainage of the medullary veins and subsequent venous infarction of the white matter. This sequence of events can sometimes be seen by sequential ultrasound examinations, when there is a change from a normal image, to simple GMH-IVH and then involvement of the parenchyma on the following day. This type of lesion is generally globular and usually communicates with the lateral ventricle with subsequent evolution into a porencephalic cyst. Recently this pattern has been shown to be more variable, with a discrete parenchymal lesion, which does not necessarily communicate with the lateral ventricle and can evolve into a few small cysts in the white matter that may resolve with eventual ex-vacuo dilatation of the lateral ventricle on the affected side. These white matter cysts can be misinterpreted as unilateral cystic leukomalacia, but seeing the evolution of this unilateral lesion on sequential ultrasound examinations would not favour leukomalacia, which is almost invariably bilateral and not often associated with a large IVH. MRI performed later in infancy or early childhood will also help to make a distinction between the two conditions, showing more focal injury in those with a unilateral parenchymal hemorrhage. Detailed studies by Dudink et al [44] have made

it possible to identify the veins involved in the parenchymal injury. Bassan et al [45] have suggested grading the parenchymal lesion, taking account of the extent of the lesion, the presence of a contralateral parenchymal lesion and the presence of a midline shift due to this lesion. This grading system has predicted neurodevelopmental outcome at 2 years of age [46].

138.6.1 Intracerebellar Hemorrhage

An important recent development, when discussing GMH-IVH in the preterm infants, is the recognition of intracerebellar hemorrhages. This type of lesion is most often associated with a GMH-IVH and is especially common in those with a birth weight < 750 grams and gestational age below 27 weeks [47–49]. Twenty-five of the 35 infants studied by Limeroupolos et al [48] had a unilateral hemispheric hemorrhage. Of the 35 infants, 27 also had supratentorial lesions. Apnea, bradycardia and a falling hematocrit may be associated with this type of lesion. Ultrasonography can be diagnostic when the lesion is large or when the posterolateral fontanelle is used as the acoustic window [47–49]. MRI will give better definition of the extent of the lesion and will also identify punctate lesions in the cerebellar hemisphere [49]. Follow-up at a mean age of 32 months showed neurologic abnormalities in 66% of infants with isolated cerebellar hemorrhagic injury compared with 5% of control preterm infants [50]. Infants with isolated cerebellar hemorrhagic injury versus controls had severe motor disabilities (48 *vs* 0%), expressive language (42 *vs* 0%), delayed receptive language (37 *vs* 0%), and cognitive deficits (40 *vs* 0%). Preterm infants with cerebellar hemorrhagic injury and supratentorial parenchymal injury were not at overall greater risk for neurodevelopmental disabilities, although neuromotor impairment was more severe. Cerebellar atrophy without apparent cerebellar hemorrhage has also been reported as a common sequel of severe immaturity [51–53]. Srinivasan et al [54] only showed reduced cerebellar volume in preterm infants at term-equivalent age in association with supratentorial pathology such as hemorrhagic parenchymal infarction, intraventricular hemorrhage with dilation, and periventricular leukomalacia.

138.7 Management

Once the diagnosis of a GMH-IVH has been made, the immediate clinical management is similar to that of other at risk preterm infants. Optimalization of any coagulopathy, minimal handling, prevention of fluctuations in blood pressure or CO_2 levels, prevention of breathing against the ventilator may be used try to prevent extension of the initial hemorrhage. A continuous record of an amplitude-integrated EEG (aEEG) registration may help to detect subclinical seizures, which will often be subclinical and may require treatment [55].

Especially in infants with a large IVH with or without associated parenchymal hemorrhage, posthemorrhagic ventricular dilatation (PHVD) may develop over the next 10–14 days. Repeat US scans are indicated to diagnose this complication. PHVD can progress either slowly or rapidly. In two-thirds of cases, slowly progressive PHVD is followed by spontaneous arrest; in the remaining one-third of infants with PHVD, ventricular size increases rapidly over the course of days to weeks [56].

There is no agreement about whether treatment of progressive PHVD before the occurrence of clinical symptoms is beneficial to the child. Due to the large extracerebral space and the high water content of the white matter, increased ventricular size, as assessed by repeated ultrasound examinations, will precede by several weeks clinical signs, such as a rapid increase in head circumference (> 2 cm/week), diastasis of the sutures, a full fontanelle, vomiting, irritability, bradycardias and apneas. Sunsetting is only seen at a late stage. Measurements are usually taken in the coronal view at the foramen of Monro, using the ventricular width [57]. The ventricular index is the distance between the midline and the lateral border of the ventricle. Most intervention studies have taken 4 mm above the 97th percentile as a starting point for randomization. Another useful measurement is the so-called anterior horn width, taken just anterior to the thalamic notch [58]. This anterior horn width measurement does not change much with increasing maturation and should be < 3 mm; measurements > 6 mm suggest PHVD. Measuring the occipital horn in a sagittal plane can also be useful, as there can be a discrepancy between dilatation of the anterior and posterior horn. Although the angle of insonation may be more variable in the sagittal plane, any measurement of the occipital horn ≥ 25 mm also suggests severe PHVD. Assessing the shape of the lateral ventricles may be useful, when making a distinction between pressure driven PHVD and ex-vacuo dilatation following white matter injury. Measuring the cerebrospinal fluid pressure may also aid in making a distinction, but reliable measurements can be hard to obtain [59].

Adverse short-term effects on the central nervous system have been shown using different techniques, including evoked potentials, aEEG, Doppler ultrasonography and near infrared spectroscopy (NIRS) [60, 61]. Cerebrospinal fluid (CSF) measurements of cytokines, non-protein bound iron, hypoxanthine and soluble anti-apoptotic factor sFas have all been found to be raised in infants with PHVD and especially so in those with associated periventricular leukomalacia [62–65]. In spite of these data, there is no direct evidence that early drainage of CSF alters the natural and long-term outcome of children with PHVD.

In the two largest randomized controlled trials (RCTs) so far, randomization was done once the 97th percentile + 4 mm line was crossed and 60% of the infants in both arms subsequently required shunt placement [66, 67]. The RCT for DRIFT (drainage intervention fibrinolytic therapy) also used this measurement as an entry point for the study [68]. The

initial data were promising with shunt requirement in 22%, but the RCT was stopped early, due to a high risk (33%) of rebleeding in the DRIFT group, without an apparent positive effect on the need for a ventriculo-peritoneal shunt [69]. In two retrospective observational studies, a significant reduction in the need for shunt placement was seen when intervention was started before this line was crossed and when the threshold for inserting a subcutaneous reservoir was low [70, 71]. Whether earlier and more active intervention is effective is now being studied by a prospective RCT.

138.7.1 Outcome Following GMH-IVH

Most studies of infants with mild grades of GMH-IVH (hemorrhage restricted to the germinal matrix or small amount of ventricular blood) suggest that these infants perform as well in cognitive and motor developmental outcome as preterm infants with no GMH-IVH, although a lower score was found with regard to their visual-motor integration [72]. Recent neonatal 3D-volumetric imaging studies, however, have shown reduced gray matter volumes at term equivalent age [73–75] associated with a reduced mental developmental index on the Bayley scale for infant development, 2nd edition (BSID-II), but this was only seen in the most immature infants with gestation below 30 weeks. Whether associated mild white matter abnormalities were present and could have played a role is not discussed in these reports. This association was previously suggested by Kuban et al [76].

The term ventriculomegaly (VM) has been used for infants with ventricular enlargement following a GMH-IVH, but also for those without apparent preceding hemorrhage when it is more likely to be due to white matter loss. It is therefore preferable to use VM to refer to babies without an apparent preceding large GMH-IVH and to use the term posthemorrhagic ventricular dilatation (PHVD) when ventricular enlargement follows a large hemorrhage [76, 77]. Sequential ultrasound examinations and consideration of the shape of the ventricles enables this important distinction in most cases.

The risk of a poor outcome has been reported to increase significantly with the presence of PHVD following a large GMH-IVH (40–60%) and even further in those who require shunt insertion (75–88%). [66, 78, 79]. In a cohort from Sweden, associated problems (cerebral visual impairment, epilepsy and especially cognitive problems) were very common [79]. In a retrospective hospital-based population study, outcome was better than reported previously: cerebral palsy occurred in only 7% of infants with a large IVH (grade III) compared to 49% of the 76 infants with a parenchymal hemorrhage ($p < 0.001$). The mean developmental quotient (DQ) in the grade III group was 99; if there was parenchymal involvement, DQ was 95 at 24 months' corrected age [61]. Whether this better outcome was related to earlier treatment of PHVD or due to a cohort with more localized lesions and of higher GA needs to be confirmed and a prospective randomized controlled trial is now underway.

While infants without apparent parenchymal involvement are more likely to develop diplegia, those with a venous infarct are at risk of developing a hemiplegia. It has been suggested that outcome with unilateral parenchymal hemorrhage varies and depends on the extent and site of the lesion [46, 80] although others have not found the site of the lesion to be predictive [81]. A recent score reported by Bassan et al showed an association between a higher score, based on the presence of a more extensive lesion, a midline shift, or bilateral parenchymal involvement and mortality and outcome at 2 years of age [46]. Long-term follow-up into childhood and adolescence showed that most children with a parenchymal hemorrhage and subsequent porencephaly were ambulatory, but required learning assistance in school and had social challenges [82, 83].

Early prediction of development of a hemiplegia is now possible using MRI at 40–42 weeks. Myelination of the posterior limb of the internal capsule (PLIC) should be present at term equivalent age. In infants who go on to develop a hemiplegia, asymmetry and even lack of myelination of the PLIC was noted in those who subsequently developed a hemiplegia [84, 85]. Using diffusion tensor imaging, visualization of the tracts is possible at an earlier stage, but data are only available in preterm born children studied in childhood looking at thalamo-cortical connectivity [86, 87].

138.8 Prevention of GMH-IVH in the Preterm Infant

Most studies have shown a reduction in the incidence of GMH-IVH over time. Both prenatal and postnatal pharmacologic prophylaxis has been used to reduce the incidence of GMH-IVH. Many different drugs have been used, such as phenobarbital, tranexamic acid, pancuronium, etamsylate, vitamin E, and indomethacin. Only a few of these will be discussed here in more detail.

138.8.1 Antenatal Prevention

138.8.1.1 Antenatal Steroids

Antenatal administration of corticosteroids has been shown by several studies to be the most important protective factor for development of GMH-IVH [88, 89]. A systematic review of 21 randomized controlled trials involving over 4000 babies has shown that corticosteroid administration is associated with a significant reduction in the risk of GMH-IVH (OR 0.54; CI 0.43, 0.69) and with a strong trend towards improving long-term neurologic outcome in survivors (OR 0.64; CI 0.14, 2.98)

[89]. The effect of steroid administration could be due to a reduction in risk and severity of respiratory distress syndrome, postnatal stabilization of blood pressure, or maybe even a direct cerebral protective effect. Repeated courses of antenatal corticosteroids are not recommended as a negative effect on brain growth has been shown [90]. without a further reduction of the incidences of GMH-IVH or leukomalacia.[91] Bethamethasone instead of dexamethasone is recommended as the latter has been associated with an increased incidence of PVL [92].

138.8.1.2 Magnesium Sulphate

Antenatal administration of magnesium sulphate was not associated with a reduction in the incidence of GMH-IVH in a large randomized multi-centre study [93]. A meta-analysis showed that antenatal magnesium sulphate therapy given to women at risk of preterm birth substantially reduced the risk of cerebral palsy in their children (relative risk [RR] 0.69; 95% confidence interval [CI] 0.54–0.87). The number needed to treat to prevent one case of cerebral palsy was 63 (95% CI 43–155). Moreover, there was a significant reduction in the rate of substantial gross motor dysfunction (RR 0.61; 95% CI 0.44–0.85) [94].

138.8.1.3 Vitamin K

Vitamin K has been used antenatally to prevent neonatal GMH-IVH, as vitamin K-dependent factors are deficient in preterm infants. Although initial reports were promising, this was not supported by a recent systemic review of 9 randomized studies involving 1750 women to evaluate the role of vitamin K in the prevention of GMH-IVH, given to women in labor or very likely to deliver a premature infant. Only two trials were of sufficient quality to be used for the final analysis [95–97].

138.9 Postnatal Prevention

138.9.1 Phenobarbital

The rationale for phenobarbital use was sedation of the preterm infant to prevent the fluctuations of blood pressure that occur with clinical care of high-risk infants. Phenobarbital was the first drug used postnatally in the prevention of GMH-IVH. A meta-analysis of 10 trials was unable to show a reduction in the incidence or severity of GMH-IVH (relative risk 1.04, 95% CI 0.87, 1.25), severe IVH (relative risk 0.91, 95% CI 0.66, 1.24) [98]. There was a consistent trend in the trials towards increased use of mechanical ventilation in the phenobarbital treated group, which was supported by the meta-analysis (typical relative risk 1.18, 95% CI 1.06, 1.32).

138.9.2 Indomethacin

A meta-analysis of the postnatal use of indomethacin in the prevention of GMH-IVH and subsequent brain injury showed a significant reduction in the incidence of GMH-IVH of all grades in indomethacin treated groups (RR 0.88; CI 0.80, 0.96) [99]. When only more severe degrees of hemorrhage were reported (Papile grade III and IV) this effect was still present (RR 0.66; CI 0.53, 0.82).

Outcome measures of death or severe neurosensory impairment was reported in four studies, but no significant effect of indomethacin could be found (RR 1.02; CI 0.90, 1.15) [99, 100]. A recent post-hoc analysis of the orginal indomethacin trial suggested that boys exposed to indomethacin had significantly better outcome in verbal test scores than females, suggesting a gender specific effect [101].

138.9.3 Ibuprofen

Ibuprofen is used as an alternative to indomethacin for use in the medical management of patent ductus arteriosus and also acts by prostaglandin synthase inhibition. A recent RCT evaluated whether ibuprofen when given shortly after birth to a group of premature infants (< 28 weeks' gestation) reduced the incidence of GMH-IVH [102]. Ibuprofen did not reduce the incidence of any degree of GMH-IVH when compared to controls (OR 0.96 CI 0.48, 2.03) or more severe GMH-IVH (grade II–IV) (OR 0.87 CI 0.25, 3.05) compared with controls.

138.9.4 General Measurements

Paying closer attention to blood pressure, gentle handling, synchronous ventilation and less severe respiratory distress syndrome due to antenatal and postnatal surfactant therapy was associated with a reduction in GMH-IVH [103]. Data from the Canadian network also showed that the incidence and severity of GMH-IVH is affected by NICU characteristics. A high patient volume and a high neonatologist/staff ratio was associated with a lower rate of severe IVH [104].

138.10 IVH in the Full-term Infant

Intraventricular hemorrhage (IVH) is an uncommon problem in full-term as compared to preterm neonates. Origins of the IVH can be the germinal matrix, choroid plexus, or parenchyma. The latter was recently reported as hemorrhagic parenchymal infarction [105]. In term infants only remnants of the germinal matrix remain. The incidence of germinal matrix hemorrhage is therefore considered to be low, but the true incidence is not really known as infants are usually asymptomatic.

The mechanism of IVH in term infants has been attributed to trauma at birth (precipitous delivery) or hypoxia; however, no etiology is detected in most cases. Recently, thalamic hemorrhage associated with an intraventricular hemorrhage was recognized to be related to cerebral sinovenous thrombosis (CSVT) involving the straight sinus [106, 107]. The pathogenesis of thalamic hemorrhage is hemorrhagic venous infarction of the large venous channels that are in close proximity to the ventricular walls. In the majority of infants none of the previously associated risk factors for thalamic hemorrhage were noted, such as coagulation disorders or hypoxic-ischemic birth injury. Predisposing factors noted by Roland et al [108] included sepsis, cyanotic heart disease, and polycythemia. Physical signs (irritability, seizures, apnea, bulging fontanel) occurred later than in infants with IVH due to choroid plexus or germinal matrix hemorrhage. Anticoagulation therapy may be considered to prevent propagation of the thrombosis [107].

Management of IVH in term infants is supportive. Prognosis depends on the location and extent of the underlying insult. As a rule, among infants for whom no etiology of the IVH is detected, outcome appears to be good. Neurodevelopmental sequelae are seen in infants with IVH with parenchymal involvement [109]. When bilateral thalamic hemorrhage is associated with birth asphyxia, mortality is high and sequelae in survivors are high. Thalamic hemorrhage with IVH seen in infants with an uneventful birth history is associated with a greater risk for cerebral palsy than IVH from other sites.

138.10.1 Subdural Hemorrhage in Term Infants

Subdural and subarachnoid hemorrhages are probably under diagnosed as they are difficult to recognize using cranial ultrasound. Subdural haemorrhage usually occurs secondary to birth trauma. These hemorrhages are relatively uncommon currently because of improvements in obstetric care. Vaginal breech deliveries are for instance less common since recent multicenter randomized studies [110]. Occipital diastasis can occur during a vaginal breech delivery with excessive extension of the neck of the infant. The pathogenesis is secondary to mechanical injury to the cranium associated with instrumental delivery with forceps or Ventouse extraction of the head, abnormal presentation (face or brow), precipitous delivery, and a large infant resulting in a difficult delivery, although others did not find an increased risk with assisted vaginal delivery. MRI scanning showed a prevalence of 26% following vaginal birth in a group of 97 infants [111–113]. Shearing forces act on the tentorium and the deep venous system. Children are usually born at term and present with a full fontanelle, lethargy, apnea and or seizures. Hydrocephalus may develop due to outflow obstruction and temporary external drainage, sometimes followed by permanent drainage, due to impaired CSF reabsorption at the level of the arachnoid granulations. Secondary cerebral infarction has also been has been related to prolonged arterial compression [114]. The majority of subdural hemorrhages are infratentorial but a supratentorial location can also be seen and is then sometimes associated with a lobar hemorrhage, which can be large and associated with a shift of the midline, requiring neurosurgical intervention (Fig. 138.4). Short-term outcome in children with an isolated infratentorial subdural hemorrhage, but also in those with a lobar hemorrhage has often been reported to be more favorable than expected, but the groups studied have been small [112, 115].

138.10.2 Subgaleal Hemorrhage in Term Infants

Subgaleal hemorrhage is a rare and potentially fatal condition of the neonate often associated with instrumental delivery. It is caused by rupture of the emissary veins, which are connections between the dural sinuses and the scalp veins. Blood accumulates between the epicranial aponeurosis of the scalp and the periosteum. Most cases of subgaleal hemorrhage reported have been associated with the use of the vacuum extractor. The incidence of subgaleal hemorrhage is estimated to occur in 4–6 of 10,000 spontaneous vaginal deliveries and in 46–59 of 10,000 vacuum-assisted deliveries [116, 117]. Children present with diffuse swelling of the head and evidence of hypovolaemic shock. In a study by Kilani et al [118] associated intracranial hemorrhage was present in half of the 34 infants studied and four died. In another study 31% had a poor outcome (five died, four had epilepsy, three with severe auditory dysfunction, two with cerebral palsy, and one with renal vein thrombosis) [119]. The group with the poor outcome had significantly more patients who had been transferred from other hospitals ($P < 0.001$). Those with a poor outcome had significantly more hypotension ($P < 0.001$) and seizures ($P < 0.05$). Prompt and aggressive administration of blood products and treatment of an associated coagulopathy are recommended to improve outcome.

138.11 ICH in Other Specific Conditions in Full-term Infants

138.11.1 Neonatal Alloimmune Thrombocytopenia

In neonatal alloimmune thrombocytopenia (NAITP), fetal and neonatal thrombocytopenia results from the formation of a maternal antiplatelet antibody to a paternally derived platelet antigen, usually platelet surface antigen (PLAI) and expressed on the surface of the fetal platelets. Neonatal

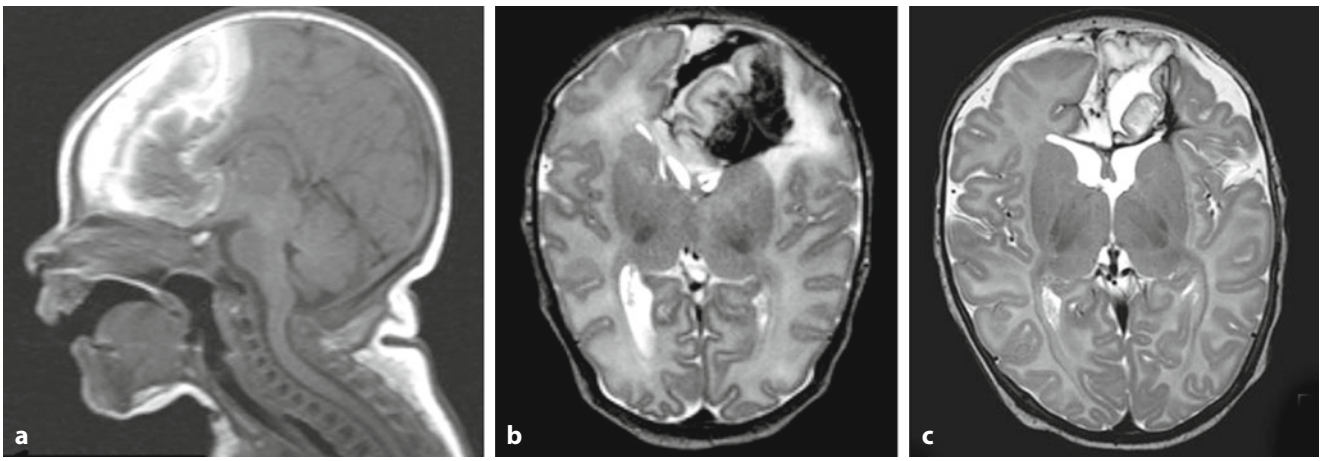

Fig. 138.4 Full-term infant, easy vaginal delivery, admitted with neonatal seizures. MRI on day 7 (**a** and **b**) and at 3 months (**c**), showing a supratentorial subdural hemorrhage and a large frontal intraparenchymal hemorrhage, with a shift of the midline. A repeat scan at 3 months, shows resolution of the hemorrhage and associated loss of brain tissue

alloimune thrombocytopenia occurs in 1 in 2000 to 1 in 5000 fetuses. ICH occurs in as many as 10–30% of the infants with alloimmune thrombocytopenia, 25–50% of which occur in utero [120]. NAITP has an estimated mortality rate of 15%, with ICH accounting for most deaths. Fetal and neonatal platelet counts below 20 x 109 are common even before 24 weeks' gestation and repeated episodes of antenatal hemorrhage have been reported [121]. Management in the antenatal period includes the administration of intravenous gammaglobulin to the mother with or without corticosteroids prior to delivery. Transfusion of matched compatible platelets to the fetus may protect against ICH during the birthing process. Cesarean section is suggested if cordocentesis reveals fetal thrombocytopenia. After birth, transfusion with antigen-negative platelets (maternal platelets) is recommended [122]. A hemispheric porencephalic cyst, following a hemorrhage which is most commonly located within a temporal lobe is usually present at birth; extra-axial hemorrhages, intraventricular hemorrhage, acute parenchymal hemorrhage, and neuronal migrational disorder have also been reported [120].

138.11.2 Extracorporeal Membrane Oxygenation

Extracorporeal membrane oxygenation (ECMO) is the treatment of choice for infants with persistent pulmonary hypertension and cardiorespiratory failure unresponsive to inhaled nitric oxide.

In term infants, ICH following ECMO occurs in 10–13% of infants [123–124] (see Chapter 72).

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Neonatal Arterial Stroke

Daniela Ricci and Eugenio Mercuri

139.1 Introduction

The term neonatal arterial stroke is used to define focal ischemic brain injuries occurring in the distribution of the territory of one (or more) cerebral arteries. Recently the term perinatal arterial ischemic stroke (PAIS) has been coined to define a group of heterogeneous conditions in which focal disruption of cerebral blood flow occurred between 20 weeks of fetal life through the 28th postnatal day [1] and confirmed by neuroimaging or neuropathological studies.

Until recently, arterial ischemic stroke was considered an uncommon condition in the neonatal period, frequently associated with motor and cognitive deficits. Lesions were usually identified on imaging obtained after the child presented with hemiplegia or epilepsy in early infancy [2, 3]. The wider availability of neonatal cranial ultrasound (cUS) and magnetic resonance imaging (MRI) has increased the detection of such lesions in the neonatal period and has changed our knowledge on their incidence and on the modality of presentation in both term and preterm infants. It is now generally agreed that stroke is the second most common cause for neonatal seizures after hypoxic-ischemic encephalopathy [4]. Recent studies report that perinatal stroke is 17 times more frequent then those occurring later in childhood [5]. Perinatal arterial ischemic stroke is estimate to occur in around 1/2,300 to 1/5,000 term neonates [6, 7] with the great majority presenting acutely, usually with seizures within 72 hours after birth, but without focal deficits or encephalopathy [8].

There have been several attempts to classify arterial strokes, with most classifications being based either on the extent of the lesions or on the timing of the insult.

In 2007 in a workshop on ischemic perinatal stroke, a classification of PAIS based on gestational age or postnatal age at diagnosis was suggested: 1) fetal ischemic stroke, diagnosed before birth by using fetal imaging methods or in stillbirths on the basis of neuropathologic examinations; 2) neonatal ischemic stroke, diagnosed after birth and before the 28th postnatal day (including preterm infants); 3) presumed perinatal ischemic stroke (PPERI), diagnosed in infants aged > 28 days in whom it is presumed (but not certain) that the ischemic event occurred some time between the 20th week of fetal life through the 28th postnatal day [1, 7].

More recently Govaert and colleagues [9], using the same classification, proposed a slightly different division within the subgroups, according to the timing of stroke, considering that lesions detected in the newborn period that presumably preceded labour should be considered separately from those that occurred during or just after labour.

They also divided infants with neonatal stroke into two subgroups by age of presentation of the symptoms. An early presentation, within the first 3 days of birth, is likely to be related to labor or delivery while a late neonatal presentation (between 4 and 28 days after birth) can be related to disorder of the late neonatal period including cardiac disease, extracorporeal membrane oxygenation, postnatal infection or other postnatal causes.

Other attempts at classification have been based on the extent of the lesion, according to the distribution of the lesion. Lesions in the territory of the middle cerebral artery (MCA) are the most common and they occur much more commonly in the left than in the right hemisphere. MCA have been further subdivided into: main branch, cortical branches, lenticulostriate branches (Fig. 139.1) [10]. This classification has been extensively used for both diagnostic and prognostic purposes.

Main branch MCA involvement is the lesion most easily detected on cranial ultrasound while infarctions in the anterior and posterior regions and smaller branch MCA infarcts can be more difficult to visualize [11, 12].

In our experience, in a cohort of over 60 cases of full-term infants with perinatal arterial lesions detected in the first days after birth, the great majority had lesions in the cortical or main branch of the MCA and only a minority had lesions in

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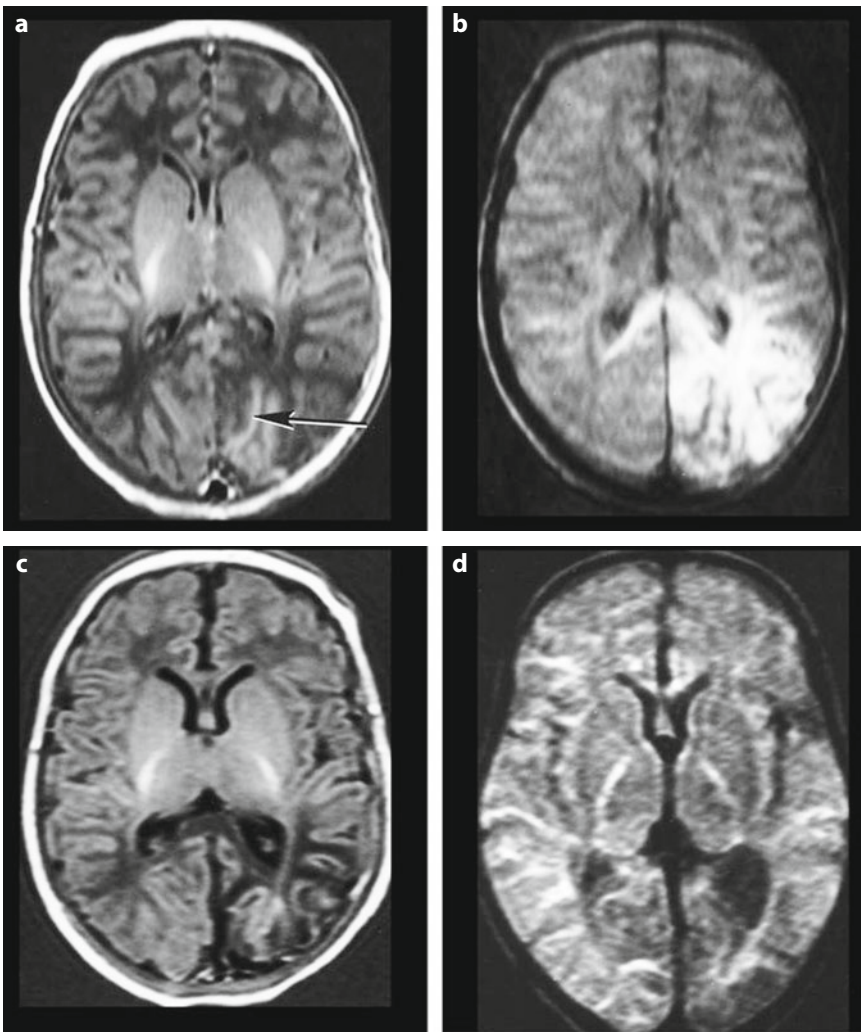

Fig. 139.1 T1 weighted images (*left*) and diffusion weighted images (*right*) performed on day 3 (*top*) and day 9 (*bottom*). Note that in the first scans the abnormalities are more obvious on diffusion (**b**) than on conventional imaging (**a**), while they become more obvious on the conventional imaging on day 9 (**c**) when they are barely visible on diffusion (**d**)

the lenticulostriate branch only or in the anterior or posterior cerebral artery.

In contrast DeVries and colleagues have reported that, in preterm infants, the lenticulostriate branches appears to be more frequently involved than in full-term infants. Until recently, most of the published studies have reported PAIS in full-term infants. In the last few years however, there has been a sharp increase in the studies reporting PAIS in preterm infants suggesting that such lesions may be more frequent than initially assumed. Bender and colleagues [6, 7] reported an incidence of 7 in 1,000 admissions in their neonatal intensive care unit. The gestational age of the infants was less than 35 weeks. They hypothesized that the higher incidence of PAIS in preterm infants might be explained by the routine use of cranial ultrasound in all the preterm infants admitted to the unit, even in those without any clinical symptoms, and the high exposure to invasive procedures.

These new findings suggest that the occurrence of strokes in units where there is not such careful ultrasound monitoring may be underestimated.

139.2 Etiology of Perinatal Arterial Stroke

Although an association between stroke and hypoxic-ischemic encephalopathy undoubtedly exists [13], in our experience it is uncommon. Possible etiological factors involved in neonatal stroke have been extensively investigated and different factors have been thought to be potentially involved in determining strokes in a number of cases [14, 15]. However, none of the individual factors reported so far appear to be consistently involved in all the cases studied [15]. Furthermore, when individual factors are assessed in a cohort of strokes and in controls several risk factors are generally present in both groups even though are significantly more frequent in the study group than in controls [16].

These findings strongly suggest not only that there is a multiplicity of etiological factors, but also that none of them, when considered separately, can be considered as the only factor responsible for the lesion. It has now been accepted that it is more likely that the risk of lesions increases when

multiple factors are present [15, 17] with a possible co-occurrence of prenatal, perinatal and other factors further increasing the risk of developing the insult and the lesion.

For preterm infants data in the literature are scarcer but some data have become recently available [18]. Particular attention has been devoted to antenatal or perinatal predisposing factors and to prothrombotic and coagulation abnormalities.

139.3 Maternal Factors

In our own studies [19] most term infants with focal infarction are born to primigravidas and this was also a risk factor in the study of Lee et al [17]. In a recently study of a cohort of proven perinatal strokes, maternal primiparity, infertility and cocaine use, were reported risk factors in term infants compared to controls [15]. Wu et al [16] also reported a significant association between perinatal strokes and prepartum risk factors such as pre-eclampsia and intrauterine growth restriction.

In infants with AIS labor is often prolonged and the delivery complicated, with emergency cesarean section and vacuum extraction significantly higher than in controls [17, 19]. In the study of Wu et al [16], newborns with PAS were at higher risk of delivery complications, such as emergency cesarean section, 5-minute Apgar < 7, and resuscitation at birth. Abnormal cardiotocograph, instrumental deliveries and emergency cesarean sections have also been reported to be significantly higher in another cohort of perinatal strokes [15]. Intrapartum complications, such as vacuum extraction, emergency cesarean section, ventouse delivery, use of oxytocin and fetal heart rate abnormality, have recently also been found to be significantly higher in preterm infants with AIS, compared to matched controls [18].

139.4 Prothrombotic Abnormalities

In the last decade we and others have suggested and reported a significant association between neonatal stroke and thromboembolic disorders [15, 20–29]. In our cohort, 10 of 24 (42%) neonates with stroke of perinatal origin had at least one prothrombotic risk factor. Five were heterozygous for the factor V Leiden (G1691A) mutation (FVL) and six had high levels of factor VIIIc [23]. Günther et al [20] also found that 68% of 91 term neonates with PAS had at least one prothrombotic factor compared with 24% of 182 controls. The most common finding was an increase in the level of Lp(a) FVL mutation in the PT gene and protein C in neonatal stroke in combination with other perinatal risk factors.

Hogeveen et al [30] found homocysteine levels to be higher in neonates with stroke, compared with healthy age-matched controls. However, in a recent study, there was no evidence for an excess of any candidate gene polymorphisms [31].

There remains much scope for research into prothrombotic abnormalities in neonatal stroke and thrombosis in the newborn infant. A recent review suggests that when combining arterial stokes and thrombosis in neonates, although prothrombotic coagulation factors are present in more than half of the cases, they are individually unlikely to play a major role in the pathogenesis of PAIS [32].

139.5 Other Factors

Sepsis, disseminated intravascular coagulation, polycythemia [33], congenital heart disease [34], and the need for vascular cannulation (all frequent occurrences in sick newborns), as well as extracorporeal membrane oxygenation (ECMO) and endocarditis, have definite associations with stroke, although these problems are more commonly seen in the preterm infant or in specialist units and we have rarely seen them in our own term patients.

Circulatory factors such as patent foramen ovale [35, 36], atrial septal defect [37] or aging placenta [38, 39] have also been reported.

139.6 Clinical Presentation

In the past strokes were mainly found in neonates with asphyxia or with severe clinical signs associated with cardiac or infective disorders. In a number of cases the presenting signs were hemiplegia or other neurological signs occurring in infants and children well beyond the neonatal period in infants who had evidence of focal lesions that were classified as presumed neonatal strokes in the absence of postnatal acute events.

More recently it has been established that in full-term infants neonatal seizures, generally occurring in the first 24–72 hours after birth, are the commonest first clinical sign in infants who were born with normal Apgar scores and were on the postnatal wards with their mothers [19]. Neurological examination may be normal, but as infants with seizures are often given anticonvulsants, they may present with generalised hypotonia [19]. Asymmetry of tone pattern may be noted but these are usually transient and are not predictive of hemiplegia at a later age [19]. This presentation is common among the infants in whom the lesion occurs perinatally but has also been reported with prenatal lesions. Low Apgar scores and neurological signs of asphyxia have been reported [13] but are less common.

In preterm infants symptoms such as clinical seizures or apneas, are frequent in preterm infants due to other underlying problems and the diagnosis is generally made on cUS but could be delayed or missed if these are not performed routinely and accurately [7].

139.7 Diagnosis

The first evidence of a lesion generally comes from US scans, even though it should be kept in mind that if the scans are performed soon after the onset of symptoms these may not be sufficient to provide a diagnosis [40–43]. In contrast, scans performed at the end of the first week usually provide evidence of focal changes suggestive of infarction. In our recent study of 47 infants who had early (day 1–3) and later (day 4–14) ultrasound we found that the early US scans were abnormal in 68% of the infants whereas the late CUS scans were abnormal in 87%, even though they were not always accurate to predict the extension of the lesion. Specific site and lesion diagnosis and identifying additional smaller peripheral lesions are better seen on MRI, which is the preferred method of diagnosis and also provides prognostic information. As for US, early MRI scans using T1 and T2-weighted images do not always identify early signal change. Most lesions can be detected on both US and MRI within 24–48 hours from seizure onset and are more clearly seen on T2 than T1-weighted images, but a proportion of lesions may be missed if the scans are performed within one or two days from the onset of seizures. In contrast, using diffusion weighted imaging (DWI), all lesions can be clearly observed even a few hours after the seizures (Fig. 139.2) [41, 42, 44, 45]. The DWI becomes less abnormal 5–7 days after birth, at the time when the signal on conventional T1 and T2-weighted images become more clearly abnormal. This pattern suggests that the insult has occurred recently, around the perinatal period. Diffusion restriction in the corticospinal tracts is an early sign of pre-Wallerian degeneration and can be used to predict outcome [46–48]. On conventional imaging, Wallerian degeneration in the brain stem can be seen as persisting abnormal signal intensity with atrophy appearing over 5–8 weeks and it is generally associated with the development of contralateral hemiplegia [49].

T1 and T2-weighted images allow the identification of the vascular distribution of the lesion and the extent of involvement of cortical and subcortical structures, such as the internal capsule, basal ganglia and also secondary changes in the brainstem and thalami or in the contralateral hemisphere that have been reported in a proportion of cases with arterial infarcts [19, 50].

The combination of very early and sequential ultrasound, and conventional and diffusion weighted MRI in the first few days after symptom onset is the most helpful to detect the lesion and to obtain information on the extent and the timing of the lesion. It has also been suggested that electroencephalography (EEG) performed soon after seizures may help in suggesting the diagnosis of stroke. More specifically, the occurrence of periodic lateralized epileptiform discharges (PLEDs) can help to suspect a focal lesion [51]. EEG has also been used as a prognostic indicator with the persistence of abnormal background activity rather than the occurrence of seizures being associated with a poor motor prognosis [19, 52].

139.8 Prognosis

While in the past stokes were mainly detected in patients who had severe clinical signs at birth or who were referred for epilepsy or motor sequelae, in the last two decades a better surveillance of neonatal seizures and the increased recognition of smaller strokes in the neonatal period has led to the identification of a number of lesions that are not always associated with abnormal outcome [53]. The issue of prognosis is however still controversial [14, 32].

The reported outcome data of neonatal stroke, however, provides very different rates of normal and abnormal outcome, depending on the inclusion criteria and on the study

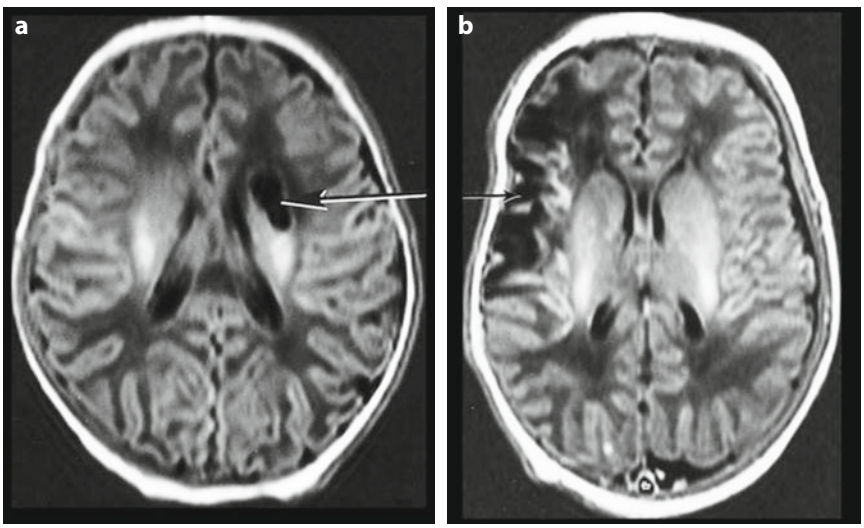

Fig. 139.2 Early brain MRI showing examples of middle cerebral artery infarcts involving the lenticulostriate branch (**a**) and the main branch (**b**)

populations. While most studies mainly include perinatal arterial infarcts in term infants, other include preterm infants, and others also those who present late with symptoms and are regarded as presumed neonatal. Because of this, comparisons of the different data sets are often difficult.

Most of the studies report short-term outcomes and mainly focus on motor outcome. Hemiplegia is the most frequently reported sequela [53] but its incidence, for the reasons given is very variable [54–64]. In our cohort of term-born infants mainly presenting with early seizures, less than a third develop a hemiplegia [19, 53, 65]. The first clinical signs of hemiplegia, when present, can be detected between 3 and 6 months and consist of mild asymmetry or abnormal patterns of movements [49, 66] and generally become more evident between 6 and 12 months [49]. In a few infants with evidence of prenatal lesions, however, clear signs of hemiplegia could already be detected in the first few months.

As not all the neonatal strokes are associated with development of hemiplegia, it is important to identify early signs which predict outcome. In our experience, neither the neonatal clinical examination nor the neonatal cranial ultrasound was helpful in prognosis [19]. Neonatal brain MRI in contrast provided better prognostic information. In our cohort motor outcome did not appear to depend on the vascular distribution of the lesion while the concomitant involvement of hemispheric tissue, the posterior limb of the internal capsule (PLIC) and the basal ganglia was always associated with an abnormal motor outcome. The involvement of only one or two of the three regions in contrast was associated with a normal outcome [19, 67]. In more recent studies [46, 48] it has also been reported that early acute DWI changes in the corticospinal tracts also predict hemiplegia.

De Vries et al [7] have recently reported that in preterm infants hemiplegia is more frequent than in full-term infants with similar lesions.

We have also found that abnormal background EEG activity in the neonatal period was also associated with abnormal motor outcome whilst a normal background, with or without seizure activity, was related to normal outcome [19].

Hemiplegia is, however, not the only possible sequela of strokes and other studies have reported the occurrence of language, vision and behavioral abnormalities and the risk of developing seizures.

139.8.1 Cognitive Outcome

In general children with neonatal stroke are thought to have a better cognitive outcome than those who suffered more global perinatal insults. Because of the site of the lesions there has been concern of the possible association between parietal lesions and language and more generally, cognitive development. In the Golomb et al [68] series, children with cerebral palsy after perinatal stroke who had neonatal presentation were more likely to have severe cognitive impairment than children with delayed presentation. In our own studies, which included all infants with neonatal infarcts and not only those with hemiplegia, however, over 80% of school age children were in a mainstream school setting with an IQ in the normal range [65]. Speech delay has been observed in children more commonly with left hemisphere infarcts [69–71] but it has been recently suggested that these early abnormalities tend to recover in the course of development [72] and that there is no evidence of decline in cognitive function over time in children with perinatal unilateral brain damage [73].

139.8.2 Visual Development

As most strokes involve the middle cerebral artery and therefore spare the occipital lobes and often also the occipital radiations, it is not surprising that visual field abnormalities are unusual. Early abnormalities of visual fields may be related to the method of assessment that also include a shift of attention, with a mechanism involving the parietal lobes. Acuity is generally normal but and other aspects of abnormal visual processing (fixation shifts, orientation-reversal visual evoked potentials [VEP]) can be found in children with neonatal focal infarction when assessed in the first years after birth [74 orientation-reversal VEP 76], even though they may recover by school age [77].

139.8.3 Psychological and Behavioral Problems

Psychological and behavioral problems have been extensively studied by Goodman [78], who finds problems with behavior, emotions and relationships in 50% of children with hemiplegia.

139.8.4 Seizures

It should also been mentioned that seizures have been reported to be common in children who suffered neonatal infarction [58, 60, 69], especially in those who developed hemiplegia. The incidence of later seizures in those who do not develop hemiplegia is low but in most studies follow up is relatively short-term [19, 40, 65, 79, 80]. It has been recently been reported that the presence of seizure may limit the chance of plasticity of the developing brain [73].

139.9 Treatment

At present there is no specific acute therapy for neonatal stroke. As strokes generally occur in infants with normal

Apgar scores in whom the only clinical sign is the onset of seizures several hours after birth, there is generally no need for intensive care, ventilation, or other types of support. Clinical seizures should be treated if they last longer than 5 minutes and occur on two occasions.

As there is little evidence of a recurrence of neonatal stroke either in the child or in the family, there is generally the tendency to avoid treatment in newborns with stroke [29]. All infants should have coagulation and thrombophilic studies and treatment should be reserved for those with abnormalities requiring it.

Anticoagulants are generally not used as there is concern about bleeding into infarcted tissue, unless there are other problems such as cardiac abnormalities or thrombi that might lead to further stroke.

Acknowledgments

The authors thank Mary Rutherford and Frances Cowan, Hammersmith Hospital, London, for their help and for providing the figures.

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140

Neonatal Seizures

Malcolm Levene

140.1 Introduction

Seizures are more common during the neonatal period than at any other time of life. This is because the neonatal brain is in a hyper-excitable state with an imbalance between the activities of neurotransmitters. During the early neonatal period, immature neurons express mainly GABA-nergic activity and paradoxically at this age GABA exerts a neuroexcitatory action. This appears to change in the early weeks of life when GABA receptors develop a more inhibitory effect [1]. As a result of this the neonatal brain is more likely to seize as a result of a variety of causes.

Clinical seizures in the neonatal period are often a dramatic symptom of brain compromise demanding urgent investigation and management. Unfortunately it is now apparent that clinically evident seizures may not reflect cortical malfunction and conversely electrographic seizures are not necessarily manifest by abnormal neurological behaviour, the so-called electro-clinical dissociation. A number of studies have shown that only between 30–50% of electroconvulsive seizures are detected clinically [2, 3]. Furthermore the management of neonatal seizures is also not clear as there is evidence that traditional anticonvulsant drugs do not stop seizures and that these drugs may actually be neurotoxic in therapeutic dosage regimens. Consequently the whole area of neonatal seizures must be revisited with a view to a reasonable clinical approach to this problem.

A recent development in the investigation of neonatal seizures are monitors that record digitally electrocortical activity and display it at the baby's bedside in a way that can be interpreted by clinical staff with basic training in EEG interpretation. This has allowed a more rational basis for investigation and management of neonatal convulsions.

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140.2 Definition of Seizure Activity

The term fits, convulsions and seizures are often used interchangeably to describe the phenomenon of the clinical manifestations of abnormal electrocortical activity. Volpe [4] defines a seizure as "a paroxysmal alteration in neurological function (ie behavioural, motor or autonomic function)." These clinical manifestations are expected to be accompanied by abnormal electrical activity recorded from scalp electrodes which are synchronous in time with the abnormal clinical manifestation, but it is now well recognized that clinical manifestations may occur in isolation to epileptic cortical electrical discharges. It is possible that the origin of these abnormal clinical manifestations may be from subcortical structures but this is difficult to show in a clinical setting. Unfortunately, it is now recognized that the clinical manifestations can be very varied and change even within the same infant in a relatively short period of time and stereotypic movements which are thought to be seizures may not be associated with abnormal electrocortical activity. To make the discussion even more complicated, it is now well recognized from video-EEG studies that neonates can show extensive electrical seizures, even amounting to status epilepticus without abnormal clinical manifestations.

As a result of this, two definitions of seizure activity need to be considered; clinical and electroseizure and there may not be synchronicity between these two types. Clinical seizures are only a symptom and should be confirmed by EEG monitoring.

140.3 Clinical Classification

The clinical manifestations of neonatal seizures have been well described, but there is no internationally agreed classification system. Clinical seizures present in a number of different ways and may vary considerably in the same baby over a number of hours. These main patterns are shown in Table 140.1 along with their relative frequency.

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 140.1 Frequency and causes of clinically evident seizures

Seizure type	Frequency*	Specific causes
Clonic	60%	HIE Cerebral artery infarction Metabolic encephalopathy
Tonic	20%	HIE Severe IVH
Myoclonic	5%	Metabolic encephalopathy Midazolam infusion HIE
Subtle	15%	Particularly seen in preterm infants HIE

* Data adapted from [5].
HIE hypoxic-ischemic encephalopathy, *IVH* intraventricular hemorrhage.

140.3.1 Clonic Seizures

These are reported to be the most frequently identified form of seizure in term infants [5]. Clonic seizures are more commonly seen in older children and comprise regular rhythmic jerking of limbs or face with a frequency of 1–3 cycles per second. They are often fragmentary and may be focal or multifocal in nature. Focal clonic seizures are particularly seen in term babies as a result of a focal neuropathology (e.g., middle cerebral artery infarction) but may be seen also in a variety of metabolic disorders. Multifocal clonic seizures may affect different parts of the body in a rapidly progressive manner although a Jacksonian type migration in the newborn is very rare. More commonly beats of clonic activity is seen in a limb, which fades in frequency to be replaced by similar abnormal movements in another limb or face. This may repeat itself frequently in the same patient. It is the most common clinical type which is associated with synchronous abnormal EEG.

140.3.2 Tonic Seizures

This refers to stiffening of a muscle group(s) for several seconds followed by slow relaxation. Limbs, face, neck or trunk involvement may occur. Involvement of the back muscles may cause opisthotonic posturing to occur. It is often associated with simultaneous deviation of the eyes. The tonic seizure may be focal or generalized. Tonic seizures are often associated with either abnormal background EEG features or simultaneous synchronous EEG activity. These movements should be distinguished from hyperekplexia where similar movements are seen but always as a result of stimulation.

140.3.3 Myoclonic Seizures

This refers to a short lasting contraction of a muscle group most commonly in the limbs and resembles twitching movements. They are not uncommon in normal babies during sleep and if they do not occur during wakeful periods are usually benign. These seizures may occur frequently but are often seen in isolation to a synchronous abnormal EEG discharge.

140.3.4 Subtle Seizures

Subtle abnormal movements in neonates are often characterised as subtle seizures, and this classification is the most variable of the above groups. Almost any repetitive and stereotypical movement can be considered as a manifestation of subtle seizures. These include sucking movements, chewing, tongue thrusting, transient eye deviation, pedalling or swimming-type movements, apnea (rare as an isolated phenomenon in term babies), transient bradycardic episodes and hiccoughs.

Subtle abnormal movements are more commonly seen in preterm infants and are often not associated with synchronous abnormal EEG activity which makes diagnosis of these movements difficult. In term infants abnormal EEG activity, either synchronous with the abnormal movements or as an abnormal background trace, is much more commonly seen.

140.3.5 Differential Diagnosis

The most common movement pattern confused with seizures are jittery movements. These are very common in preterm infants and may be due to hypocalcemia or hypomagnesemia. The frequency of jitteriness movements is significantly higher than the 1–3 cycle per second of clonic convulsive movements. The onset of jitters is almost always the result of some external stimulus and can usually be inhibited by holding the affected limb whereas movements due to clonic seizures are transmitted to the examiners hand when the limb is held.

Occasional myoclonic jerks are often seen in normal sleeping babies and provided they do not occur when the baby is awake they may be described as benign neonatal sleep myoclonus. This is associated with a normal EEG.

Hyperekplexia is a rare genetic condition (usually autosomal dominant) where babies have a very exaggerated response to external stimuli such that they have generalised muscle contractions which may be confused with myoclonic epilepsy. Patients with hyperekplexia (also called congenital stiff-man syndrome) have a normal EEG and usually have a family history. A molecular marker has been described for this condition [6].

140.4 EEG Classification

Traditionally formal EEG assessment has presented considerably logistic and diagnostic difficulties in neonatal intensive

care units for a number of reasons. The technique requires considerable technical skills to apply electrodes correctly, to ensure appropriately low electrode impedance, expert diagnostic interpretation of traces from an experienced neurophysiologist, the potentially hostile environment of a neonatal unit with regards to electrical interference and the practicality of being able to access the technique on all occasions that a baby shows possible convulsive activity. This has largely put the routine use of this technique out of reach for all but a few neonatal intensive care units in the acute situation. A more recent development has been the introduction of video-EEG where a more continuous digital EEG signal is acquired from the baby simultaneously with a continuous video recording of the babies' movements. The two outputs can then be displayed side by side on one screen so that correlation can be made retrospectively between potentially abnormal movements and EEG activity. This technique has proven very important in understanding the correlation of unusual clinical features with synchronous discharge activity of the EEG and also in recognizing the extent of electroclinical dissociation in the newborn infant, but it is of very limited value in routine clinical practice.

Particular problems in the interpretation of the neonatal EEG arise as a result of the considerable maturation of the EEG pattern in preterm infants progressively up to term and beyond. A description of this maturation is beyond the scope of this chapter but is reviewed by Mizrahi [7] and Hellstrom Westas [8]. For these reasons, recording of a highly modified and compressed continuous EEG signal has been developed and this is referred to as amplitude integrated EEG (aEEG) or cerebral function monitoring (CFM). Newer machines have attempted to integrate raw EEG signals with a compressed aEEG trace displayed in real-time on a semi-logarithmic scale and displayed on the monitor by a baby's cot-side. The majority of cot-side machines display a signal from a single pair of electrodes placed in a biparietal distribution with a midline reference electrode. aEEG is valuable in recognizing seizure activity and abnormal background activity (Fig. 140.1) and has been used as a criterion for entry into studies assessing neuroprotection in term asphyxiated babies [9].

The limitation of aEEG monitoring is that of very localized acquisition of EEG from a single pair of electrodes. The aEEG is particularly unreliable in the presence of multi-focal or localized seizure activity and seizure activity of short duration. In a study comparing aEEG with continuous video EEG recording, approximately half of all neonatal seizures detected by video-EEG were missed by aEEG [10]. Nevertheless aEEG is now increasingly used as a method of brain monitoring in neonatal intensive care units. Providing its limitations are recognized and suspicious traces are evaluated by full EEG assessment, it is likely to be a useful technique.

140.4.1 Status Epilepticus

There is no internationally accepted definition of status epilepticus in the newborn and it is considered to be a rare condition in preterm infants. One definition is continuous electro-convulsive activity in the newborn lasting 30 minutes or more or comprising > 50% of the EEG record [11, 12]. Boylan et al [13] have shown that continuous seizure activity lasting for 30 minutes is rare in the neonate, and have suggested that repeated seizure activity for 15–30 minutes in a 1 hour period represents a high-risk appearance for poor outcome.

140.5 Etiology of Neonatal Seizures

The incidence of clinically evident neonatal seizures has been variously reported to be 1.8–3.5 per 1000 live births [14–16], but in very immature babies (< 1500 g) this was considerably higher at 19–57.5 per 1000 [15, 16]. This data should be treated with caution because there are no studies of a large group of EEG diagnosed seizures in the newborn and there was little consistency in the studies about the definition of clinical seizures.

There are many causes of neonatal seizures and the more carefully a baby is investigated the more likely it is that a cause will be identified. In some cases no cause is found (idiopathic group) and these babies often have a good prognosis. Table 140.2 lists the most common causes of neonatal

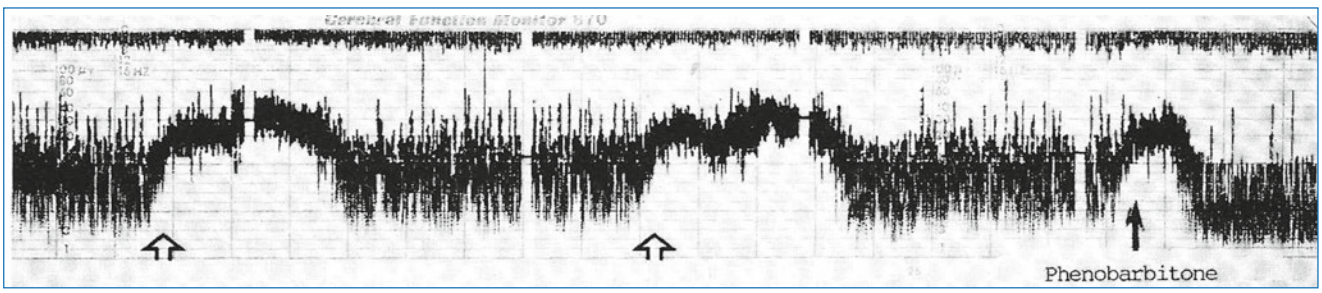

Fig. 140.1 Cerebral function monitor (aEEG) trace showing two convulsions (*wide arrows*) and the effect of phenobarbitone infusion during a third convulsion in a full-term infant. Note also that the voltage background trace between the first two convulsions is abnormally low

Table 140.2 Cause, incidence and outcome of neonatal convulsions in term infants [32]

Etiological factors	Incidence (%)	Poor outcome (%)*
Asphyxia	40	50
Cerebral arteriovenous infarction	15	0
Intracranial hemorrhage	15	13
Congenital cerebral anomaly	3	100
Hypoglycemia/Hypocalcenmia	7	**
Infection	10	**
Inborn errors of metabolism	1	**
Unknown cause	10	0

* Refers to moderate and severe neurodevelopmental abnormality.
** Numbers too small to make meaningful predictions.

seizures together with incidence and outcome for each group. The following represent the main causation groupings.

140.5.1 Hypoxic-Ischemic Encephalopathy

Approximately one half of all infants who develop neonatal seizures have hypoxic-ischaemic encephalopathy (HIE) as the underlying cause. This condition can only be reliably recognized in term infants although infants of 35 and 36 weeks' gestational age may often show at least partial features of HIE. The incidence of HIE is 3–4 per 1000 full-term infants but seizures only occur in 1–2 per 1000 of these. Seizures develop in the first 24 hours of life and in some severe cases may be present within the first 3 hours after the hypoxic-ischemic insult. The seizures usually persist for 2–7 days although in the most severe cases seizure activity may continue for weeks or months. The most commonly observed seizures in these babies are clonic or subtle, particularly with swimming, doggy-paddling or pedalling-type abnormal movements.

140.5.2 Cerebral Artery Infarction

Infarction of a major cerebral artery, usually the middle cerebral artery, occurs in 4 per 1000 babies and the most common clinical manifestation are focal clonic seizures. Characteristically these babies appear to be neurologically normal between seizures although asymmetry of tone is often seen in unilateral middle cerebral artery infarction. Diagnosis is made on magnetic resonance (MR) brain scan, particularly on diffusion weighted sequences in the early stages of the disease evolution. Ultrasound is a much less reliable method of making the diagnosis.

Sinovenous thrombosis is increasingly recognized and may present with neonatal seizures particularly if the thrombosis causes intracranial hemorrhage, most commonly in the region of the thalamus.

140.5.3 Intracranial Hemorrhage

Neonatal seizures are much less commonly seen in very immature infants than in those at term, consequently although abnormal electroconvulsive activity may be present on EEG evaluation, clinical seizures are not evident or only partial or subtle in type. Therefore, although germinal matrix hemorrhage-intraventricular hemorrhage (GMH-IVH) is common in very immature infants, these babies do not commonly present with seizures. Large parenchymal venous infarction secondary to GMH-IVH may present with clinical seizures (usually tonic) but in my experience this is uncommon.

Other forms of hemorrhage which may cause neonatal seizures include subarachnoid, subdural or less commonly cerebellar hemorrhage. A subdural hematoma in a term infant more commonly is associated with seizures than a similar hemorrhage in premature infants. Large subarachnoid hemorrhages are reported to commonly present with neonatal seizures, but it may be difficult to separate the subarachnoid hemorrhage from underlying hypoxic-ischemic brain injury. It is reported that babies with large subarachnoid hemorrhage (SAH) show normal interseizure behavior.

140.5.4 Infection

Meningitis may present with neonatal seizures although these babies usually show non-specific abnormalities prior to the onset of seizures. The incidence of neonatal meningitis is 0.25–0.5 per 1000 babies and the commonest bacterial cause is group B beta hemolytic streptococcus. Other causes of brain infection including encephalitis which may cause neonatal seizures include cytomegalovirus, toxoplasma and herpes simplex. Lumbar puncture is indicated in all cases where neonatal meningitis/encephalitis is suspected. Management should include broad spectrum antibiotics and acyclovir intravenously.

140.6 Metabolic

140.6.1 Hypoglycemia

Hypoglycemia is an important avoidable cause of brain damage. Symptomatic hypoglycemia is associated with a variety of non-specific neurological signs; seizures are seen in about 50% of those with symptoms. Blood glucose assessment should be included in the work-up of all neurologically abnormal neonates and any hypoglycemia rapidly treated. Persistent hypoglycemia may be due to hyperinsulinism. Adverse neurodevelopmental outcome is reported in up to 50% of hypoglycemic infants who develop neonatal convulsions. Hypoglycemia may lead to encephalopathy with continuing convulsions after the biochemical hypoglycemia has been corrected.

140.6.2 Hyponatremia/Hypernatremia

Another relatively common metabolic cause of neonatal convulsions include severe hyponatremia particularly when this occurs rapidly as differences between intracerebral and extracerebral osmolality across the blood-brain barrier result in brain swelling. Similarly rapid development of hypernatremia may result in brain shrinkage and neonatal convulsions possibly induced by secondary subdural hemorrhage. Rehydration should be introduced slowly to prevent rapid shifts in intracellular water content.

140.6.3 Hypoglycemia/Hypomagnesemia

Convulsions associated with hypocalcemia occurs in the first few days of life. These babies usually respond rapidly to intravenous calcium infusion. The prognosis is usually good. Persistent hypocalcemia indicates the need for careful investigation of maternal calcium metabolism and infant parathyroid function.

Late onset hypocalcemia occurs much less frequently than in the past due to enhanced phosphate supplementation in infant formula feed preparations.

Hypomagnesemia can also provoke a condition which resembles neonatal seizures but this is rare and is managed by additional magnesium and calcium infusion.

140.7 Congenital Brain Anomalies

Congenital brain anomalies, particularly those affecting cortical development are commonly associated with neonatal convulsions and may present with abnormal fetal movements which are subsequently shown to be convulsive in nature. The most common abnormality associated with the development of seizures in the neonatal period is neuronal migration disorder including pachygyria, lissencephaly and micropolygyria. Seizures due to these conditions more commonly occur after 3 days of age but may have their onset at any time in infancy.

140.8 Inborn Errors of Metabolism

Some inborn errors of metabolism (IEMs) commonly present with neonatal encephalopathy and neonatal seizures [17]. These infants usually present once milk feeding has commenced, but a few disorders present early before feeding is established. The most common IEMs causing neonatal encephalopathy and seizures are listed in Table 140.3.

Table 140.3 Metabolic disorders most commonly associated with neonatal seizures

Early onset of seizures in neonatal period
Amino Acid disorders
– Non-ketotic hyperglycinemia
– Pyridoxine-dependent seizures
– Biotinodase deficiency
– Menkes syndrome
Late onset of seizures in neonatal period
Branched-chain organic acidurias
– Maple syrup urine disease
– Proprionic aciduria
– Isovaleric aciduria
– Methylmalonic acidemia

140.8.1 Vitamin-Dependent Seizures

As a group of causes of neonatal seizures these are very rare, but an important group because early recognition and effective treatment will prevent protracted seizures into older life and will reduce brain injury.

140.8.2 Pyridoxine-Dependent Epilepsy

This is an autosomal recessive disorder associated with very early onset of seizures. Sometimes the mother describes abnormal movements felt before the baby is born. Although biochemical markers have been described, the diagnosis is usually made in infants with refractory seizures who respond to intravenous pyridoxine infusion. Although there is no specific EEG marker for this condition, there is usually a marked change in the EEG activity during infusion. Treatment is with life-long pyridoxine (vitamin B6) replacement therapy.

140.8.3 Pyridoxamine Phosphate Oxidase Deficiency

More recently a deficiency in the pyridoximine enzyme pathway has been described which normally regulates dopamine and serotonin pathways. Deficiency causes intractable seizures which is responsive to pyridoxal phosphate infusion.

140.8.4 Folinic Acid-Response Seizures

Presentation is in the first few days of life with seizures intractable to traditional anticonvulsant drugs. The seizures respond rapidly to infusion with folinic acid (2.5–5 mg twice a day), but later recurrence may occur requiring an increased dose of folinic acid.

140.9 Other Causes

140.9.1 Drug Withdrawal

Seizures are rarely seen in neonatal abstinence syndrome, although less specific abnormal neurological signs are common. Methadone, heroin, cocaine, tricyclic antidepressant and benzodiazepine drugs given to the mother or taken illicitly during pregnancy may all be associated with neonatal abstinence syndrome. The onset of seizures usually occurs between day 1–7 after birth.

140.9.2 Benign Familial Neonatal Convulsions

This disorder is due to an autosomal dominant gene with high penetrance. Multiple generations are usually affected in the same family and the prognosis is good with resolution of seizures after a few months. Between seizures the baby appears neurologically normal. Mutations have been reported in the long arms of chromosomes 20 and 8 which determines voltage-gated K^+ channels.

140.9.3 Early Myoclonic Encephalopathy

This is a severe form of recurrent myoclonic and sometimes clonic seizures which usually start by 2–3 weeks of life. It is associated with a characteristically abnormal EEG trace worsening during sleep. It may be due to an inborn error of metabolism. Seizure control with anticonvulsive drugs is difficult and prognosis is poor.

140.9.4 Otohara Syndrome

The condition, also called early infantile epileptic encephalopathy presents with multiple tonic spasms in the first few weeks of life. The EEG shows initially a burst suppression pattern, later evolving to a very disordered pattern similar to hypsarrthymia. Otohara syndrome may be associated with cerebral dysgenesis. The prognosis is very poor and is usually associated with early death.

140.10 Idiopathic

After very careful investigation, no cause can be found for the seizures in some infants. The frequency of so-called idiopathic (probably better referred to as cryptogenic) depends on the extent to which investigations have been pursued. Generally if no cause is found after careful investigation the prognosis is good particularly if the baby remains neurologically normal between seizures.

140.10.1 Fifth-day Fits

This is also referred to as benign idiopathic neonatal seizures and has been described in most parts of the world. The infant develops seizures at between 4–6 days of life having shown quite normal neurological behavior up to the time that the seizures are first recognized. The seizures are usually multifocal clonic and rarely persist beyond 24 hours although they may be frequent during that time. Cerebrospinal fluid (CSF) zinc deficiency has been described in some cases. The prognosis is good and if the diagnosis can be confidently made anticonvulsant medication is probably not required.

140.11 Management

140.11.1 Do Seizures Cause Brain Damage?

The answer to this question determines how aggressively management with antiepileptic drugs should be pursued. Understanding of this question has been recently informed by experiments in immature animals which have attempted to model short but frequent seizures as occurs commonly in the neonate. Older studies looking at the effect of prolonged status epilepticus in mature animals have little relevance on understanding the neonatal situation. Frequent short-lived electroconvulsive seizures were produced in neonatal rat pups by brief periods of fluorothyl inhalation exposure. The usual paradigm was to induce 5 seizures for 5 consecutive days and then examine the brain for neuropathological evidence of damage and to test the surviving animals for measures of cognitive function [18, 19]. The investigators had shown that a single prolonged neonatal seizure did not produce neuronal injury.

This model of multiple short seizures did not produce any neuronal damage evident at examination under the microscope when the neonatal brain was sacrificed, but if the animal was allowed to survive into adult life and then the brain examined there was clear evidence of morphological change including cortical neuronal activation and changes in cell density [18]. In surviving adult rats that were subject to short but frequent neonatal seizures they showed learning and memory deficits compared with control animals. In support of this, when status epilepticus was induced in adolescent animals who had been previously exposed to short seizures in the neonatal period, neuronal necrosis resulted compared with controls subjected to adolescent status but no neonatal convulsions [19]. It appears that neonatal seizures may prime the developing brain to damage if a subsequent insult occurs.

In summary there is now good data from immature animal studies suggesting that although neonatal seizures do not cause neuronal necrosis, neurological function may be adversely affected with long-term adverse effects. Consequently, the control of neonatal seizures by antiepileptic drugs may be more important than previously believed. Unfortunately the current choice of antiepileptic drugs (AEDs) for neonatal seizures have not been shown to be particularly effective in reducing the burden of seizure activity and concern is mounting whether these drugs may cause additional brain injury.

140.11.2 Antiepileptic Drugs for Neonatal Seizures

If there is a specific cause identified for the seizures then specific therapy is indicated such as intravenous glucose for hypoglycemic convulsions. If there is no specific treatment or when seizures continue despite appropriate specific therapy (e.g., hypoglycemic encephalopathy), then antiepileptic drugs (AEDs) are usually indicated. The two most widely used AEDs in the neonatal period are phenobarbitone and phenytoin.

There have been very few randomized controlled trials of phenobarbitone and the few that have been done do not show a high response rate to this drug. Painter et al [20] compared the efficacies of two AEDs, phenobarbitone and phenytoin, in a group of full-term infants with EEG diagnosed seizure activity. The study design randomly allocated each fitting baby to receive either phenobarbitone or phenytoin to achieve free plasma concentration levels in the therapeutic range. If the infant continued to have significant seizure activity the other drug was added to the regimen. In less than 50% of either group were the seizures completely controlled on the first drug and in only 59% was adequate control achieved when both drugs were used together. Those babies that responded best to the first line drug were those with the least frequent seizures registered on the EEG.

In another EEG study of neonatal seizures where continuous video and EEG were co-registered the authors [21] showed that only four of 14 infants responded to phenobarbitone (20–40 mg/kg) and in the other ten babies EEG evident seizures actually increased although the clinically evident seizure activity reduced. This electro-clinical dissociation is a worrying feature of phenobarbitone use as clinical staff may be given a false sense of security by a reduction in the clinical seizure burden. Those babies that responded best to phenobarbitone were those with the least abnormal background EEG trace. Two other studies [22, 23] evaluated phenobaribitone treatment in babies with seizures secondary to HIE. Hall [22] showed that high dose phenobarbitone (mean dosage 39 mg/kg) compared with standard dose (mean 27 mg/kg) reduced clinical seizure activity and subsequently showed a significant reduction in death and disability (RR 0.30, CI 0.10–0.93) but this benefit has not been confirmed in a Cochrane meta-analysis [24]. Singh et al [23] also showed a reduction in clinically evident seizures following administration of phenobarbitone. These results may reflect a reduction in clinical seizures rather than electroconvulsive seizures as shown by Boylan et al [21].

Midazolam has been evaluated as a second or third line AED in neonates who fail to respond adequately to phenobarbitone or phenytoin infusion [25]. Castro Conde et al [26] used midazolam (iv bolus of 0.15 mg/kg, followed by continuous infusion (1 μg/kg/min) increasing by 0.5 to 1 μg/kg/min every 2 minutes until either a favorable response had been achieved or the dosage reached 18 μg/kg/min). A favorable response was defined as no more than two electrical seizures lasting < 30 seconds per hour. They showed that in 10 of the 13 neonates given midazolam for status epilepticus, the infusion successfully stopped seizure activity although some required a second bolus infusion before seizures were controlled.

Lidocaine is also used in some European countries as a second/third line anticonvulsant. Malingre et al [27] reported the effects of this drug on 20 neonates with ongoing electroconvulsive seizures refractory to phenobarbitone and midazolam. Abolition of seizures was achieved in 76% of the treatment courses and no baby was reported to have developed a cardiac arrhythmia as a result of the lidocaine infusion. Cardiac arrhythmia as a result of lidocaine infusion is thought to be a particular risk if the baby is pretreated with phenytoin and this combination of drugs should be avoided. The dosage regimen is shown in Table 140.4.

Table 140.4 AED dosage regimens for use in the neonatal period

Drug	Loading dose	Second loading	Maintenance dose
Phenobarbitone	20 mg/kg by slow iv infusion over 20 min	10 mg/kg	2.5–5 mg/kg/per day
Phenytoin*	20 mg/kg by slow iv infusion over 20 min	Not recommended	
Midazolam	150–200 μg/kg iv infusion	1 μg/kg/min iv infusion increasing to 5 μg/kg/min until appropriate response	
Lidocaine*	2 mg/kg iv over 10 min	6 mg/kg/hr for 6 hr	then 4 mg/kg/hr for 12 hr, then 2 mg/kg/hr for 12 hr
Lorazepam	0.05–0.1 mg/kg by slow iv injection		
Diazepam	0.3–0.4 mg/kg iv over 3–5 min		

* Phenytoin should not be used with lidocaine.

There are few scientific data on the use of other anticonvulsants in the management of neonatal seizures although a number of benzodiazepines (diazepam, clonazepam, lorazepam) have been used in an uncontrolled manner for many years. Thiopental has been used to treat intractable seizures in the newborn, but is associated with a worrying reduction in blood pressure.

140.11.3 Do AEDs Cause Brain Damage?

A further dimension to consider in the conundrum of managing neonatal seizures concerns the risks that AEDs may cause neuronal damage in addition to any damage secondary to the seizures themselves. Phenobarbitone and phenytoin stop seizure activity in only 50% of patients as first line AEDs and complications of these drugs have been reported including inhibition of brain growth [28], neuronal toxicity [29] and adverse behavioral and cognitive effects lasting into adult life [30].

There are also reports that AEDs used in doses which produce serum levels not uncommonly seen in the neonatal period cause neuronal apoptosis in neonatal rats [31]. Phenobarbitone, phenytoin, diazepam, and clonazepam all produce this effect. The use of phenobarbitone and diazepam together produced profound apoptotic neurodegeneration. This data causes anxiety that AEDs used in the neonatal period may themselves be neurotoxic and for this reason that they should be used with caution. Monotherapy is safer than using two or more of these agents together. The most resistant seizures in the newborn occur in those babies who have sustained the most severe brain insult. The combination of severe insult, prolonged seizures and multiple use of anticonvulsants is a potentially disastrous occurrence.

140.12 Prognosis

The prognosis of neonatal seizures depends predominately on the underlying cause of the seizures. In one recent study, 72% of term infants with seizures were described as normal at 1 year of age [5]. Normal or mildly abnormal background EEG traces were associated with a favorable outcome in 89% of cases [5].Poor outcome is particularly seen in babies with asphyxia and congenital cerebral dysgenesis. The outcome is best in infants with convulsions that respond rapidly to AEDs or those in whom no cause is found after careful investigation. There is evidence that prolonged neonatal convulsions may affect brain function measurable in later life and some AEDs may themselves have a deleterious effect on reduction of neuronal numbers. About 20% of infants with neonatal seizures had seizures again after discharge from the neonatal unit [5].

The following principles should be followed in all babies with neonatal seizures:

- Carefully investigate every seizing baby including brain imaging, and metabolic screen.
- Monitor frequent seizures with aEEG to aid treatment
- Avoid using multiple AEDs whenever possible and it is preferable to use a single drug to maximum dosage
- Stop AEDs prior to discharge from hospital wherever possible.

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The Timing of Neonatal Brain Damage

Giuseppe Buonocore and Serafina Perrone

141.1 Introduction

Early abnormalities of brain development and underlying genetic factors can affect brain susceptibility to the injury [1–3]. Some infants may inherit a predisposition to have significant injury in the event of a normally sublethal insult [4]. These newly identified risk factors for brain damage in different periods make the timing of lesions important for exact diagnosis and prevention.

Incidence of neonatal encephalopathy ranges from about 2.0 to 6.0 per 1000 live births [5–7]. Hypoxic ischemic encephalopathy ranges from about 1.0 to 8.0 per 1000 live births. Epidemiological data shows that 30% of cases of neonatal encepalophaty in developed populations and 60% in developing populations have some evidence of intrapartum hypoxic-ischemia [8].

Perinatal hypoxic ischemic brain injury was demonstrated to be a major contributor to developmental disabilities in children, accounting for 25% of all cases [9].

Of the antepartum events that contribute significantly to the development of neonatal encephalopathy, fetal growth restriction ranks high. Increasingly, a strong role for fetal inflammation as one of the most frequent causes of acquired brain damage in perinatal period is being recognized.

141.2 Placental Pathology

The placenta has been described as a diary of intrauterine life and placental pathology assists in characterizing the antenatal environment. Dramatic, but at least common, are sentinel lesions, each of which can cause hypoxia sufficient to result in brain injury. There are three mechanisms for sudden catastrophic injury: (1) the placenta becomes prematurely separated from the underlying maternal uterine vascular supply: abruptio placenta or uterine rupture; (2) fetal placental blood vessels rupture; or (3) prolonged umbilical venous blood flow interruption. Placental findings that support a diagnosis of massive fetomaternal hemorrhage include a profound increase in the number of circulating nucleated red blood cells in the villous circulation, the finding of a large intervillous hematoma, villous edema, fetal arterial constriction, and villous capillary-venous dilatation. Large vessel hemorrhages generally affect vessels that are not protected by Wharton's jelly or the chorionic plate. Pathologic lesions associated with complete umbilical vessel occlusion include tight true umbilical cord knots, occlusive umbilical vein thrombi, tightly twisted (hypercoiled) umbilical cords, prolapse or tight entanglements around body parts. When this occurs, there may be an abrupt change in umbilical cord color, shape, or diameter that suggests chronicity. Other pathologic umbilical cord lesions, such as an excessively long cord length, lesser degrees of hypercoiling, abnormal cord insertion sites, and paucity of Wharton's jelly, are significantly more common in infants with cerebral palsy [10–12].

The mechanisms by which feto-placental large vessel lesions act on the vascular wall include toxic damage (meconium-associated vascular necrosis), maternal antifetal vasculitis (obliterative fetal vasculopathy), infiltration by activated fetal neutrophils (chorioamnionitis with intense fetal inflammatory response) and thrombosis (fetal thrombotic vasculopathy) [13–15]. Fetal thrombotic vasculopathy and chronic villitis with obliterative fetal vasculopathy begin weeks before delivery but continue to evolve and progress until parturition [16]. Vascular occlusion is the result of an inflammatory vasodestructive process that develops when maternal leukocytes inappropriately cross the trophoblastic barrier to enter fetal tissue. Chorioamnionitis with a severe fetal inflammatory response (intense chorionic vasculitis) and meconium-associated necrosis of vascular smooth muscle cells develop as a consequence of subacute processes beginning days before delivery [15–18]. An increased time of

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

exposure to meconium-stained amniotic fluid leads to a gradual progression of pigment through the amnion into the chorionic plate and eventually into contact with large fetal vessels.

Placentas can be abnormally small and have a decreased total gas-exchanging surface area. Alternatively, they may have decreased efficiency owing to diffuse chronic injury.

Other placental findings are markers for processes affecting either mother or fetus. In some cases, they may represent attempts to ameliorate an adverse environment; in others, they may be maladaptive consequences of it. Perhaps the most controversial of these findings is a significant increase in the number of circulating nucleated red blood cells to more than 2500/cm^3 in the fetal placental capillary circulation [19, 20]. An increase of this magnitude represents a fetal bone marrow response to hypoxia that takes 6–12 hours or more to mount [21]. A second adaptive response is a generalized increase in the number of fetal capillaries per terminal villous cross-section or chorangiosis [22, 23].

Placental pathology assists in characterizing the antenatal environment. Histological examination of the placenta is a sensitive and accurate diagnostic system for identifying lesions that can either directly cause or decrease the threshold for brain injury. The high prevalence of inadequate placental examination in cases without an identified clinical or pathologic lesion highlights the importance of a thorough placental examination in all potential cases of adverse neurologic outcomes.

141.3 Cardiotocography and Fetal Doppler Studies

Cardiotocography (CTG) is an indirect sign of fetal well-being with various technical pitfalls and a high incidence of false positives. Currently the technique is improved in the determination of the risk and timing of brain injury. In particular, the evaluation of trends and the speed of CTG changes in deceleration patterns makes it possible to distinguish harmful ischemic events from other forms of hypoxia which do not necessarily injure the fetal brain [24]. CTG combined with pulse oximetry, fetal blood pH and ECG provides further advantages [25]. Fetal electrocardiography (ECG) wave form analysis indicates the ability of the fetal heart to respond to the stress of labor. An increased T/QRS ratio identifies a fetal heart muscle that responds to hypoxia by increased utilization of heart glycogen [26]. ST segment depression may indicate a situation in which the heart does not respond fully and a negative ST may signify cardiac dysfunction and hypotension. It has been suggested that an increase in T/QRS ratio maintained for more than 10 minutes is associated with aggravation of fetal condition when intrapartum hypoxia may progress to asphyxia. Automatic analysis of CTG and ECG has been reported to enable a reduction in the incidence of newborns with severe neurological symptoms [26].

Doppler ultrasound of the blood flow in the umbilical artery (UA), descending fetal aorta (DAo), middle cerebral artery (MCA) gives insight into fetal cardiovascular responses to intrauterine growth restriction, fetal anemia, and fetal hypoxia [27]. Blood flow is classified as abnormal when pulsatility index (PI) values of MCA falling below the 5th percentile or PI value of UA, DAo, UA/MCA ratio, Dao/MCA ratio above the 95th [28]. Absent or reversed end diastolic flow of the UA, absent or retrograde net blood flow of the aortic isthmus, and the absent or retrograde end-diastolic flow of the ductus venosus are also classified as abnormal. The presence of a notch, as signals of increased perinatal vascular impedance and of brain sparing, is also highly significantly related to maternal blood serum concentration of tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) level [29]. Severe abnormalities in fetal blood flow might result in neuroanatomical or functional changes that correlate to later cognitive capacity [30].

141.4 Neuroimaging

Cranial ultrasound (US) has been used for many years to determine the type and evolution of brain damage. The ultrasound criteria of cerebral cavitation have been selected in order to give assurance that the damage may have occurred before delivery. US and post mortem studies of the evolution of brain abnormalities suggest that cystic lesions develop over 14 days [31, 32]. Serial US images have been used to study the timing of hypoxic-ischemic injury, though the reliability of US is limited by poor visualization of the subarachnoid space, cerebral cortex and posterior fossa. It is also difficult to distinguish between hemorrhagic and non hemorrhagic ischemic damage.

Conventional magnetic resonance imaging (MRI), volumetric analysis, diffusion tensor imaging (DTI) methods and surface-based morphometry (SBM) methods has recently been used to detect antenatal, perinatal and neonatal abnormalities and timing on the basis of standardized assessment of brain maturation [33, 34]. MR imaging allows reliable assessment of myelinization and establishment of a timetable for maturation and myelination of the developing brain *in vivo*.

The peak of subplate formation and function that are required for normal cortical development correlates with the peak period of vulnerability to injury in preterm infants. Injury to subplate cells has been implicated in the causation of cortical deficits in preterm infants [35, 36].

If lesions or delayed maturation of with matter (WM) disrupt specific subsets of cortico-cortical, long distance connections, these altered connectivity patterns could in turn give rise to specific abnormalities in cortical folding patterns. Thus, WM injury resulting in a secondary disruption of cortical gray matter (GM) development may be a major mediator of cognitive deficits faced by preterm infants.

Cortical development may be also altered in the absence of WM or subplate injury in preterm infants. The high sensitivity of MR is important to detect early damage occurring before reliable US imaging, particularly for WM lesions in the preterm infants.

Accurate measurement of specific cerebral tissue volumes is achieved by segmentation of the imaged volume into tissue types, followed by 3D reconstructions. Volume changes during normal maturation of cerebral tissue between 29 and 41 weeks gestation have been defined in vivo using MR tissue segmentation of the brain. Segmentation is the process wherein contours are constructed that divide the brain into the representative structures of interest depending on their signal intensity (e.g., gray matter, myelinated white matter, cerebrospinal fluid [CFS]) determined using mathematic algorihms [37].

Preterm infants with WM lesions have a 17% reduction in absolute cortical GM volume as well as a 25% reduction in absolute myelinated WM volume compared with preterm infants without WM lesions [38]. Volumetric MRI studies conducted in school aged preterm children have reported shorter corpus callosum [39, 40], hippocampus [41], basal ganglia, and amygdala [40] as well as enlarged lateral ventricles [41]. All have been shown to be associated with neuropsycological impairment.

Diffusion tensor imaging and diffusion anisotropy are based on measurement of physical caracteristics of water molecules in tissue. One parameter describing these displacements is the apparent diffusion coefficient (ADC). ADC values are commonly used clinically for the detection of acute cerebral injury.

Diffusion anisotropy provides information about the local environment.

Anisotropy values for WM show a steady increase during development, particularly when fiber tracts undergo myelination [42].

Anisotropy can also be applied to GM maturation. The loss of anisotropy associated with maturation can be used to assess cortical development and reflects varying rates of development among different cortical areas [43]. The developing cortex showed lower ACD values and anisotropy with increasing maturation, likely related to the increasing complexity in tissue organization in the mature cortex [44].

Diffusion-based MR images may be normal on the first day after injury, decrease between 2 and 4 days after injury, and return to normal after approximately 7 days [45]. MR images illustrate the natural history of acquired fetal brain lesions in relation to stage of development [46]. The detection of a single type of damage in the developing fetus is evidence of acute or chronic lesions which can be seen alone or in combination. Patterns can indicate infection or hypoxia-ischemia at various stages of intrauterine life.

Phosphorus magnetic resonance spectroscopy studies changes of cerebral oxidative metabolism during and following asphyxia [47]. Infants with neonatal encephalopathy often have normal cerebral oxidative metabolism on magnetic resonance spectroscopy shortly after birth, but many then develop delayed energy failure 6–15 h later. In survivors, the degree of secondary energy failure after 24–48 h was closely associated with neurodevelopmental impairments at 18 months and 4 years of age [48]. Sequential MRI allows demonstration that the acute brain injury associated with encephalopathy in term infants evolves progressively over time to involve initially unaffected regions [49].

141.5 Electroencephalography

The timing of brain insult can be assessed by considering serial EEG recordings findings in relation to the time of birth [50, 51]. The timing can be considered prenatal if an EEG immediately after birth already shows chronic stage abnormalities, perinatal if it shows acute stage abnormalities, and postnatal if it is normal and a later EEG shows acute stage abnormalities. The detection of even mild seizures is an important indication of timing since prolonged seizures exacerbate hypoxic brain damage [52].

Routine serial EEG studies in preterm infants demonstrated that one fourth of cerebral palsies in these infants were of antenatal origin, two thirds of perinatal origin and postnatal injuries played the smallest role. Periventricular leucomalacia (PVL) manifesting itself on the ultrasound in the late neonatal period and suggesting postnatal origin was often found to be of antenatal origin with an EEG soon after birth [51]. PVL without apparent causes was often associated with abnormal fetal heart rate patterns and early neonatal EEG abnormalities, and considered to have originated in the antepartum period.

141.6 Hematology

Nucleated red blood cell (NRBC) count indicates fetal hypoxia in term and preterm newborns. Since NRBC is physiological in cord blood of preterm neonates, single NRBC counts need to be compared with standard values for the baby's gestational age [53]. Use of NRBC as a marker of timing of asphyxia is justified by information about fetal response to hypoxia, namely increased erythropoietin (Epo) secretion due to decreased fetal PO2 [54]. A relationship between asphyxia, high levels of circulating Epo and high NRBC has been demonstrated [55]. Although the exact time elapsing between hypoxia and appearance of NRBC is not known, experimental studies showed a complex mechanism of Epo-induced late erythroid differentiation and there is evidence that a period of more than 24 hours is necessary between decreased fetal PO_2 and appearance of NRBC in blood [56]. The concept that increased NRBC in cord blood may be regarded as a marker of hypoxia for a period of more than 24

hours before birth has also been accepted on the basis of clinical observations [57, 58].

Increased lymphocyte count is considered a reliable index of brain damage and makes it possible to establish timing since the increase seems to occur approximately 25 minutes after fetal bradycardia and hypoxia [20]. Thrombocytopenia can also help to determine the timing of brain damage since it appears 20–28 hours after bradycardia. Thrombocytopenia has been confirmed to occur after fetal hypoxia although the cause is unknown [59]. Although it may be misleading in certain conditions, the number of circulating lymphocytes together with other markers such as bradycardia, time of correcting acidosis, thrombocytopenia and clinical condition may be useful in determining the time of brain lesions.

141.7 Clinical Assessment

There is no ideal measure to assess the severity of perinatal hypoxia-ischemia during the initial clinical presentation of the infant immediately after birth. A persistence of an Apgar score of 0–3 at 5 min of life is one of the criteria for the presence of perinatal asphyxia.

During the first 12–24 h of life, after moderate to severe perinatal fetal hypoxia-ischemia, signs of neonatal encephalopathy include poor tone and feeding, difficulty maintaining respiration seizures and lethargy or coma.

141.8 Biomarkers

The term biomarker can refer to any entity that occurs in the body and can be measured to predict the diagnosis, onset or progression of disease process.

Laboratory studies generally use cord blood or amniotic fluid. They provide much information about conditions jeopardizing the central nervous system. Cord blood pH was the most widely used test for additional information on the severity of intrauterine asphyxia.

The criteria promulgated by ACOG and AAP [60] to attribute intrapartum hypoxia as a cause of neonatal encephalopathy and later cerebral palsy include: (1) an umbilical arterial PH of ≤7.0, (2) early onset of moderate-to-severe neonatal encephalopathy, (3) a diagnosis of cerebral palsy with quadriparesis of the spastic or dyskinetic type, and (4) the absence of other possible causes.

When using an umbilical arterial pH at the time of birth to define intrapartum asphyxia, the incidence an umbilical arterial pH <7.0 is 3.7 of 1000 term live births. Of neonates with this degree of acidosis 17.2% survived with neonatal neurologic morbidity, 16.3% had seizures and 5.9% died during the neonatal period. In developed countries the incidence of hypoxic ischemia encephalopathy (HIE) at term is 2.5 of 1000 live births and the proportion of cases of cerebral palsy associated with intrapartum asphyxia is 14.5% [61].

Since pH is influenced by both respiratory and metabolic factors, it represents an inverse log of the hydrogen ion concentration; before it does not change linearly with hydrogen ion concentration or with the use of base. If one assumes that organ and cellular response to hypoxia-ischemia result in accumulation of acid in a relatively linear proportion to the degree and duration of the insult, the exponential function pH has limited usefulness in the prediction of hypoxia timing. On the contrary, the base excess represents a linear correlate and thus a potentially valuable index of the degree and duration of metabolic acidosis. It has been proposed that a base excess of –12 mmol/L is a threshold of a metabolic acidosis associated with newborn complication [62] while a base excess of –22 has been reported to be associated with minor or major deficit in 80% of infants with intrapartum asphyxia [63]. Following Hagelin and Leyon [64] on average, the second stage labor decreases base excess by approximately 1 mmol/L per hour in the normal fetus. Studies carried out with the comparison between base excess and prolonged heart deceleration suggest a loss of 1 mmol/L per 2 minutes of severe compromise while subacute fetal compromise may reduce the buffer base by 1 mmol/L per 6–15 minutes.

Measurement of the degree of acidosis should be associated with heart rate and blood pressure recording. In particular, unremitting bradycardia has been observed to coincide with the development of ischemic basal ganglial brain damage [65]. Studies on timing have been carried out with heart rate and pH recording at birth together with lymphocyte and thrombocyte count [66]. Neonates may have severe metabolic acidosis and disorders responsible for hypoxia-ischemia, unremitting until birth. An hypoxia-induced myocardiopathy mirrors the severity of perinatal hypoxic-ischemic insult. Myocardiopathy can lead to hypothension and hypoperfusion of important organs, such as the brain. In most infants recovery of myocardial contractility occurs in the first 24–48 h after birth [67].

The availability of rapid methods for measuring lactate in cord blood and blood of neonates suggests their extensive use for the detection of severe metabolic acidosis which reflects asphyxia and is a signal of brain damage [68]. Da Silva et al [69] showed that blood lactate concentrations over 9 mmol/L at 30 minutes of life have 84% sensitivity and 67% specificity in the prediction of moderate to severe brain damage. Values less than 5 mmol/L indicate absence of risk [70].

The occurrence of oxidative stress in cord blood appears to be a good index of severe prenatal hypoxia/asphyxia.

During hypoxia, increased production of free radicals and/or decreased detoxification lead to mitochondrial dysfunction, inhibition of protein synthesis, inhibition of adenosine-5-triphosphate synthesis, inner mitochondrial membrane damage, activation of pro-caspase and other mechanism of apoptosis, generation of nitroperoxide and increased oxidative stress [71]. Free radicals affect glial cells, consequently decreasing glutamate uptake, and N-methyl-D-aspartate receptor

dysfunction, increasing intracellular calcium and further increasing brain cell damage [72]. Free radical excess may also be responsible for endothelial dysfunction, coagulation disorders and vasoconstriction. The central nervous system is vulnerable to free radical damage due to its high content of metals which may catalyze formation of free radicals, especially hydroxyl radicals and nitroperoxide. Antioxidant defences, which are even low in the central nervous system of adults, are particularly low in fetal and neonatal oligodendroglia.

Increased oxidative stress in hypoxic fetuses and neonates can be detected by assaying serum isoprostanes, serum total hydroperoxides, advanced oxidative protein products and increased non protein bound iron (NPBI) in serum. These markers can be used for the early prediction of the risk for oxidative stress-related diseases [73]. Plasma NPBI has been found to be the best early predictive marker of neurodevelopmental outcome, with 100% sensitivity and 100% specificity for good outcome at 0–1.16 μmol/L and for poor outcome at > 15.2 μmol/L [74].

Post mortem assessment of oxidative stress has been proposed in order to clarify the origin of brain lesions. Assessment is currently made by measuring advanced glycation end products from oxidation of proteins, carbohydrates and lipids in cytoplasm, nucleus and membranes [75]. The results of studies using these methods may help to determine the timing the brain injury, since oxidation products appear to vary in relation to gestational age. By measuring isoprostanes in amniotic fluid it is possible to distinguish fetal growth restriction pregnancies from normal ones [76]. Oxidative stress in pregnancy it is also associated with preterm premature rupture of membranes (PROM) and amniotic fluid isoprostanes may be a reliable predictive index of risk of preterm PROM [77].

Considering the close relationships between free radical release and phagocyte function and the relationship between phagocyte activation and infection, additional markers of infection, particularly those of so-called remote infection, could be exploited to determine the occurrence of oxidative stress. Intrauterine infection may be followed by brain damage due to the direct effects of bacterial toxins and lipopolysaccharides on glial cells, due to astrocyte deregulation and due to the effects of phagocyte activation, particularly on coagulation and the endothelium [78]. The many observations of poor outcome in babies whose gestations were complicated by chorioamnionitis, and of high cytokines such as IL-6, IL-1β, IL-8, IL-9, TNF-α in serum of premature and full-term newborns with placental lesions typical of chorioamnionitis, suggest that it may at least be worthwhile assaying IL-6 as a marker of brain damage in utero [79, 80].

A meta-analysis of chorioamnionitis and cerebral palsy indicated a positive association among preterm and full-term infants, with a relative risk of 4.7 for the full-term infants [81]. Similarly, maternal fever was linked with an increased incidence of neonatal encephalopathy [82].

Cord blood concentrations of B-lymphocyte chemoattractants, ciliary neutrophil factor, epidermal growth factor, IL-5, IL-12, IL-13, IL-15, macrophage migration inhibitory factor, monocyte chemoattractant protein-3, monokine induced by interferon-γ and tumor necrosis factor-related apoptosis-inducing ligands have been found significantly high in preterm and full-term newborns who develop cerebral palsy. Preterm infants with cerebral palsy have higher epidermal growth factor and lower levels of granulocyte macrophage colony stimulating factor, IL-23, macrophage derived chemokine as well as pulmonary and activation regulated chemokine than matched controls [83]. IL-18 has also been identified as a marker of brain damage risk [84].

Matrix metalloproteinases (MMPs) and their inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), are microglial secreted proteins, making them easily measurable in fluids, including the peripheral blood [85, 86]. MMPs and TIMPs MMP-9 is upregulated in peripheral infracted cortex at 24–48 hours after cerebral ischemia in human forensic study in brain tissue after ischemic and hemorrhagic stroke [87]. Plasmatic levels of MMP-9 and TIMP-1 were explored in perinatal asphyxia: MMP-9 levels were significantly higher in the group of patients with unfavorable outcomes [88].

Protein S100 is brain-specific and is a very promising marker of brain damage in newborn, also providing information about timing [89].

The glial protein S100 belongs to a family of calcium binding proteins found as homo- or hetero-dimers of two different subunits (α and β). Different combinations of the subunits make up the heterodimeric forms α-α, α-β and β-β; types α-β and β-β are described as S100B protein and are shown to be highly specific for nervous tissue [90].

S100B can be measured in several biological fluids such as CSF, blood, amniotic fluid and urine.

During active brain injury at least some of the S100B released from the damaged tissue could spread into the systemic circulation as a result of hemodynamic rearrangement of the blood brain barrier.

In preterm infants with intraventricular hemorrhage (IVH), concentrations of S100 in blood were elevated before a radiological diagnosis could be made [91]. S100B blood concentrations also correlated with abnormal cerebral hemodynamic patterns (increased cerebrovascular resistance) and with the extent of IVH both in preterm and in full-term asphyxiated infants [91, 92]. In asphyxiated full-term infants, an early increase in S100B was found to be predictive of HIE and subsequent adverse neurological outcomes [93]. Longitudinal S100B protein monitoring in peripheral blood demonstrated a peak concentration of the protein 6 h after birth with a progressive decrease in S100B at 24 h. The positive predictive value of S100B for HIE with a protein cut-off of 8.5 μg/L at 2 h from birth was 71%, the negative predictive value was 90%, the sensitivity was 71%, and the specificity was 90% [93].

In intrauterine growth restriction (IUGR) fetuses a significant correlation between protein S100 and cerebral hemodynamics was observed, and high values of this protein were detected in the maternal bloodstream of pregnant women

complicated by IUGR whose newborns developed IVH [94]. S100B is also measurable in the urine fluid, and its concentrations at birth are significantly higher in preterm newborns who later developed cerebral bleeding and/or brain damage at a stage when all routine clinical, laboratory, and ultrasound investigations were still silent [95]. The very fast increase in S100 protein after hypoxic insult may be useful in determining the timing of brain injury occurring independently of enduring CTG abnormalities. Serial urine determinations of S100 protein showed increasing values from birth to 72 hours in newborns with abnormal outcome, and were higher than in babies with normal outcome. The values were reported in relation to time and severity of brain insult.

Hypoxemia is a specific trigger for an increase in activin A in the circulation of fetal lambs. Activin A is a dimeric protein belonging to the transforming growth factor-β superfamily, mainly produced by the placenta, decidua and fetal membranes and secreted in large amounts in the maternal circulation [96]. Activin A is involved in cell growth and differentiation, neuronal survival, early embryonic development, and erythropoiesis. Activin A concentrations significantly increase in maternal serum with advancing gestation [97]. Disorders of pregnancy due to reduced placental perfusion and various degrees of feto-placental hypoxemia, such as pre-eclampsia and fetal growth restriction are characterized by increased levels of maternal and umbilical cord activin A [98]. Feto-placental or maternal isocapnic hypoxemia are specific triggers for an increase in activin A.

Cord blood activin A levels increase in sheep after induction of hypoxia, remain elevated throughout hypoxia, and return to control values when normal blood flow is restored [99]. Neonates with clinical signs of perinatal hypoxia had higher activin A levels, which are correlated with biochemical features of hypoxia, such as higher nucleated red blood cell counts, plasma hypoxanthine, xanthine, base deficit levels, and lower pH [100].

Activin A concentration at birth is increased in preterm newborns who later develop IVH, and is useful for the early identification of infants with hypoxic-ischemic brain insults who are at high risk for IVH [101].

In conclusion NRBC, NPBI, isoprostanes, Activin A, protein S-100 and cytokines may be measured singularly or together to predict of brain injury earlier. The assessment of multiple biomarkers, combined with neuroimaging, has greater clinical utility for the formulation of diagnosis and the process of following diseases outcome.

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Thrombosis in the Development of Newborn Brain Damage

Paul Govaert

142.1 Introduction

Neonatal stroke is defined as a group of heterogeneous conditions in which there is focal disruption of cerebral blood flow secondary to arterial or cerebral venous thrombosis or embolization, between 20 weeks of fetal life through 28th postnatal day, and confirmed by neuroimaging or neuropathological studies [1]. Two types of ischemic neonatal strokes are arterial ischemic stroke (NAIS) and cerebral sinovenous thrombosis (CSVT). The third stroke type is primary bleeding (hemorrhagic stroke). Additional forms of ischemic stroke unique to the perinatal period include periventricular venous infarction (PVI) and presumed perinatal stroke detected in infancy or childhood (both are not discussed in this chapter). Vessels are occluded by thrombosis, embolism, direct trauma, compression, spasm or obliteration by an inflammatory process. Open vessel ischemia, e.g., during hypovolaemia, arrhythmia or asphyxia, is not included in the definition of stroke.

Neonatal arterial ischemic stroke (NAIS) can be defined by either of two methods: (i) documenting partial or complete occlusion of the artery related to a focal brain lesion; (ii) using imaging to document a lesion pattern that cannot be otherwise explained than by occlusion of a specific brain artery. Precise vascular designation [2] and site description are important in reporting perinatal stroke. Middle cerebral artery (MCA) stroke covers more than 50% of the pial strokes, complete and posterior truncal stroke being most common. Templates for the diagnosis of neonatal perforator stroke have been published, with ultrasound and magnetic resonance imaging (MRI) for MCA stroke types [3]; and with ultrasound for the other perforator stroke types [4]. Multiple separate strokes in the same infant can be due to: infarction with meningitis, embolism (including air), thrombophilia, arteriopathy, breakdown of a large thrombus into several emboli and triggered arterial spasm.

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Neonatal cerebral sinovenous thrombosis (NCSVT) is defined as the presence of thrombus in a sinus, a large deep brain vein or a smaller pial vein, with partial or complete occlusion. Complete occlusion, depending on tempo of thrombosis, hemostatic dysfunction and collateral potential, may or may not cause venous infarction (red softening) in brain areas draining into the vessel. In a minority such infarcts remain purely ischemic, suggesting that arterial occlusion in response to venous ischemia occurs prior to vein rupture. Specific hemorrhagic lesions have been reported in this context: thalamo-ventricular hemorrhage with occlusion of the internal cerebral vein (bilateral with great vein of Galen occlusion) [5], striato-hippocampal bleeding with basal vein thrombosis [6], parasagittal subcortical bleeding with superior sagittal sinus thrombosis [7], temporal lobe or cerebellar hemorrhage with transverse sinus thrombosis [8], temporal lobe anterolateral hemorrhage with tentorial sinus or temporal diploic vein rupture [9] and temporal lobe hemorrhage with vein of Labbé thrombosis [10]. Large veins and sinuses usually recanalize following thrombosis, over a period of weeks to months [11].

Hemorrhagic stroke results from rupture of an (ab)normal intracranial blood vessel, but it is difficult to document damage of a vessel in such instances. In the newborn, intracranial hemorrhage is usually described under the heading of the brain compartment affected: intraventricular (bleeding in choroid plexus and ventricle), thalamoventricular (related to internal cerebral vein occlusion), lobar cerebral (bleeding in the parenchyma of a cerebral lobe), cerebellar, subarachnoid and sub/epidural. Because sub/epidural bleeding is often due to trauma or hemostatic failure, and because intraventricular hemorrhage cannot be confused with arterial stroke, it seems appropriate to register only lobar (including subarachnoid) hematoma as stroke [12–14]. Specific types of frank hemorrhage are due to venous thrombosis (see above).

This chapter is a guide to the diagnosis of primary thrombosis in the newborn brain, be it arterial or venous. The likelihood of finding a prothrombotic condition and/or a dysfunctional platelet-endothelium interaction is probably greater

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in such instances. A systematic exclusion of mechanisms other than primary thrombosis is therefore clinically relevant.

142.2 Clinical Presentation

Seizures are the usual presentation of NAIS [15]. Onset is fifty-fifty divided between day one and later in the first week, rarely beyond day three [16]. Focal seizures are a more common first sign of NAIS than general seizures (see Chapter 139). Because the interval insult to seizures may be short (less than an hour) but also add up to several hours, the timing in hours of an arterial insult in relation to labor stages is impossible. Acute embolic stroke may cause seizures within the hour of the event [17] or onset of seizures may be delayed for several hours [18]. Some newborns present with apneic spells or cyanotic attacks, possibly of epileptic nature [19]. Most children seem to be alert between seizures and even accept oral feeding. Some present with temperature instability [20], others with bouts of hyper- or hypotension due to hypothalamic injury. In some infants hemiplegia can be predicted from neonatal appreciation of asymmetrical general movements [21], but most often future hemiplegia is inconspicuous in the neonatal period. Neurologic alarm signs are usually lacking in perforator stroke and in the preterm, where stroke is often a surprise finding at brain ultrasound (US) scanning.

In the context of NAIS an alarming event is acute pallor and loss of pulsation of (part of) a limb due to arterial embolism (or spasm ?), as reported by several authors [22–28, one personal observation]. Most of these limb-brain strokes present with limb pallor within minutes of delivery, strongly suggesting that embolic stroke preceded delivery. In some cases antiphospholipid antibodies in newborn serum were associated, and aortic thrombosis has been documented in this setting [25, 28, personal observation]. In this context subclavian steal from the vertebrobasilar circulation has been documented with MRI [28].

Neonates with NCSVT present with seizures (two thirds) or focal neurological signs like hemimotor paresis or cranial nerve palsies [29, 30]. These signs suggest that parenchymal destruction around veins followed an increase of venous tension above the arterial pressure level. Fever, altered level of consciousness or jitteriness are possible but not typical. The underlying cause may dominate the picture as with asphyxia, congenital heart disease or dehydration. Thrombosis without infarction is asymptomatic and thus an incidental finding (for instance in a search for the cause of a low platelet count or during extracorporeal membrane oxygenation [ECMO]). Platelet consumption is seen with NCSVT, with or without venous infarction, but is only seen with NAIS if the arterial process causes organ or limb necrosis [31]. Propagation of thrombosis is common in the first week, perhaps explaining evolving clinical signs [11].

142.3 Etiology, Pathogenesis and Differential Diagnosis

142.3.1 Step 1: Exclude Entities Mimicking Stroke

Several focal lesions have to be differentiated from NAIS and NCSVT: kernicterus, encephalitis (bacterial and viral), mitochondrial disorders, posterior reversible encephalopathy and tumor. Consistent imaging patterns of neonatal hypoglycemic injury have been reported. Bilateral, rather symmetrical, injury of occipital and posterior parietal cortex and underlying white matter was well documented in a postmortem study of three patients [32] and subsequently in many *in vivo* studies [33].

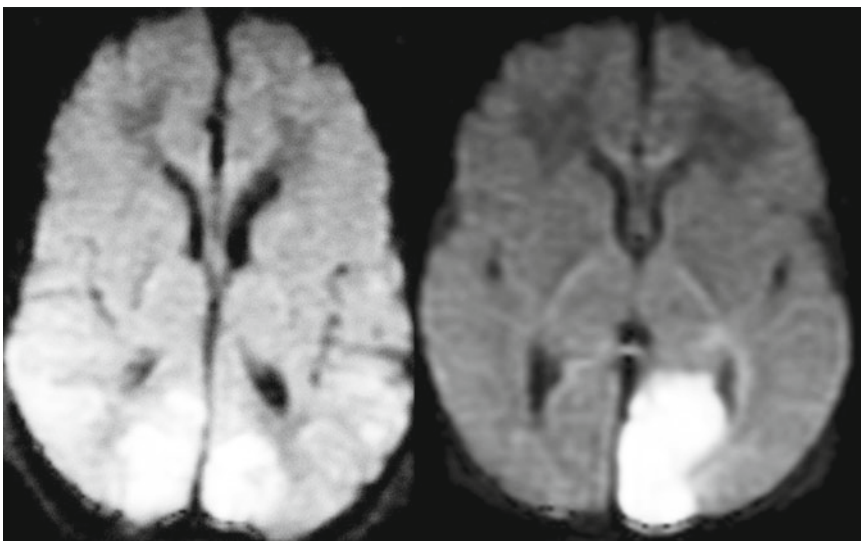

Fig. 142.1 DW axial MRI in a term infant with profound hypoglycemia and seizures: focal bilateral occipital subcortical cell injury may mimick posterior cerebral artery (PCA) stroke (compare with DWI on the right of infant with tip of the basilar artery stroke leading to cerebellar infarction and left PCA pial stroke)

Injury spreads beyond the area covered by the posterior cerebral artery and is too extensive to be classified as watershed infarction (Fig. 142.1). Infarction with cavitation, not a feature in adults, has been reported in the newborn [34]. A second paradigm seems to be unilateral hemispheric necrosis [35].

Bleeding from a vascular anomaly should be distinguished from stroke. It is advised to repeat ultrasound Doppler exams or even magnetic resonance angiography (MRA) 4–6 weeks after hemorrhagic stroke, if the cause of the insult is not obvious (no source of embolism, no infection, no trauma, normal hemostasis) [36, 37]. Developmental venous anomalies may resemble focal pial stroke in some infants.

Watershed injury resulting from partial asphyxia or hypotension (e.g., due to acute blood loss) does not fit under the stroke umbrella. However, one must be aware that hyperechoic change in parasagittal areas can be due to interarterial watershed injury but also to superior sagittal sinus thrombosis.

It is important not to confuse stroke with different degrees of bilateral white matter injury typical of the preterm (former leukomalacia, often with petechial or nodular hemorrhage in affected areas). Matters are more complicated concerning periventricular venous infarction (PVI), regularly seen in the preterm infant [38]. Such (almost invariably) unilateral white matter hemorrhage in preterm infants is in fact consequent to venous obstruction following ipsilateral germinal matrix hemorrhage or intraventricular hemorrhage (IVH), but the clinical context is different from NAIS or NCSVT. In addition, routine serial sonographic screening reliably offers recognition of PVI in the acute stage [39, 40]. Some instances will not be due to compression of a medullary collector vein by germinal matrix hemorrhage but to primary thrombosis.

142.3.2 Step 2: Recognize Hemorrhagic Stroke

142.3.2.1 Lobar Hematoma

In a limited number of newborn infants, often in the absence of IVH, one may find hemorrhage in one or more brain lobes, outside the basal ganglia and thalamus [13, 14, 41, 42]. Lesions in every lobe of the cerebrum have been described, and the prevalence amounts to about one hemorrhagic stroke for five ischemic ones (Fig. 142.2) [42]. It is common for lobar hematomas to be associated with a subdural or subarachnoid extra-axial component: this raises the question where the primary focus is, in parenchyma or in the extracerebral space. The presenting sign has often been apnea [19, 43] or (multi)focal convulsive activity, but some lobar hematomas are asymptomatic [44]. In most instances the mechanism remains unknown [12]. There are no reports of documented sinus thrombosis causing large lobar hemorrhage in the absence of a hemostatic problem [9]. The association of lobar hematoma with focal arterial ischemia in another additional brain area is suggestive of embolism with hemorrhagic conversion of arterial ischemic stroke.

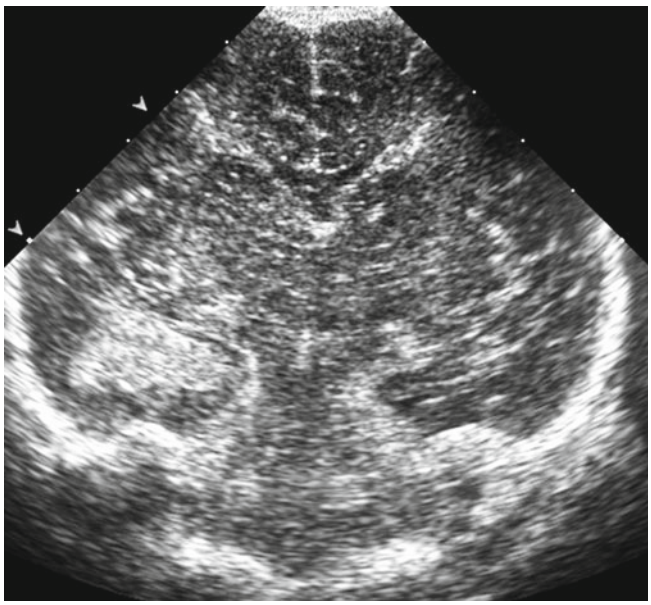

Fig. 142.2 Coronal US 6 hours after birth of a term infant presenting with apnea and cyanosis: hyperechoic right temporal lobe lesion, later demonstrated to be a hematoma; vascular malformation excluded by MRA and serial Doppler imaging

Lobar lesions also warrant a diligent search for evidence of trauma, hemostatic failure [45], a prothrombotic tendency and a vascular malformation. Saccular arterial aneurysms can present with seizures and meningeal signs in infancy: the MCA is commonly affected but location can be within the posterior fossa or in other supratentorial arteries. Most aneurysms exceede 1 cm in diameter and should be MRA-present. Arterio-venous malformations (other than vein of Galen malformation) present with congestive heart failure (usually large fistulae), hemorrhage or hydrocephalus of the communicating type. Most are supratentorial (9/10). Spontaneous thrombosis of an arteriovenous malformation (AVM) is exceptional. Cavernous angiomata are associated with hemorrhage as a rule, from mild to extensive. One must be aware of an underlying tumor facing lobar, subdural or intraventricular hemorrhage: astrocytoma is a rare cause of neonatal lobar hematoma.

142.3.2.2 Subarachnoid Hematoma

Subarachnoid hematoma refers to a space-occupying hemorrhage on the brain surface, located under the arachnoid membranes [46, 47]. The primary lesion is a fast growing subarachnoid or subpial hemorrhage [48], mostly along and under the temporal and parietal lobes, more often on the left. There is usually a context of hemorrhagic diathesis, frequently intravascular coagulation (or thrombocytopenia of any cause) during sepsis. On any imaging technique the borders of the hematoma irregularly penetrate the parenchyma

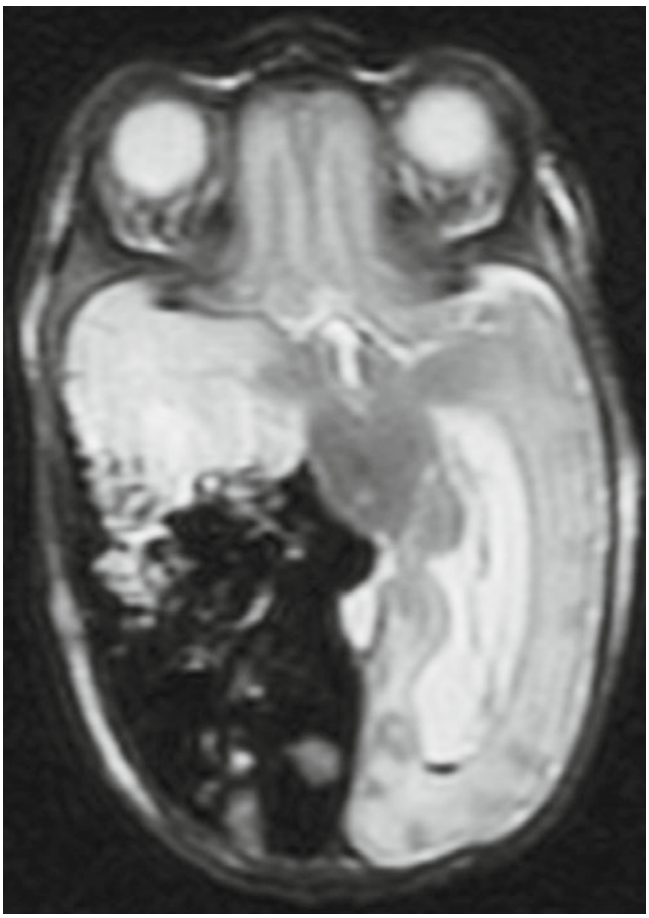

Fig. 142.3 Axial T2 MRI: subarachnoid hematoma associated with gram-negative septicemia in a preterm infant

because sulci and fissures are filled with clot (Fig. 142.3). Subdural hemorrhage displaces but does not penetrate the adjacent parenchyma. The midline may be shifted and the ipsilateral ventricle can be closed under pressure. Rupture of an arterial aneurysm or AVM is another cause of subarachnoid hematoma [49–51]. Subarachnoid hemorrhage was one of the components of a strange and often fatal pattern of hemorrhagic necrosis in both parietal lobes of extremely preterm infants [52]. These lesions cavitated in the parenchyma. Vigorous chest physiotherapy was recognized as the underlying mechanism and the situation is thereafter discussed as shaken newborn syndrome [53, 54].

142.3.3 Step 3: Link Stroke to (Birth) Trauma

There are several possibilites that explain how difficult delivery can cause vessel occlusion. Direct injury to the vertebro-basilar arterial system is possible [55]. Several reports associated difficult instrumental traction with intimal damage to the internal carotid or middle cerebral artery resulting in local occlusion from thrombosis and stroke [56–60]. Focal brain damage in the sense of contusion can be observed with bone fracture or dehiscence [61, 62]. Both PCA and superior cerebellar artery can be compressed and occluded during uncal herniation induced by lobar parenchymal, epidural or subdural hematoma [63–67]. In the vicinity of subdural or subarachnoid bleeding stroke has been tentatively (because never angiographically documented in the newborn) linked to spasm [15, 60, 62, 68, 69]. Trauma may predispose to arterial infarction outside the context of difficult delivery. During ECMO embolism from the canulated (traumatized)

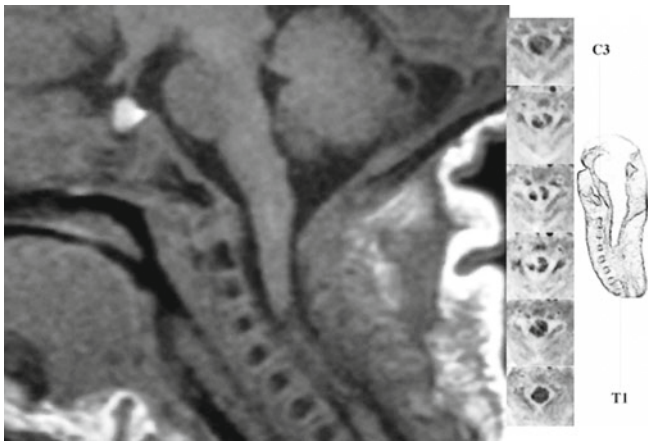

Fig. 142.4 Term boy with quadriparesis from birth (hyperextended breech for which elective cesarean) deceased due to respiratory insufficiency in early infancy. MRI (sagittal T1 with assorted T2 axial scans at different cervical levels) pointed to a lesion from C3 to T1 that divided the cervical cord into a smaller right and larger left portion

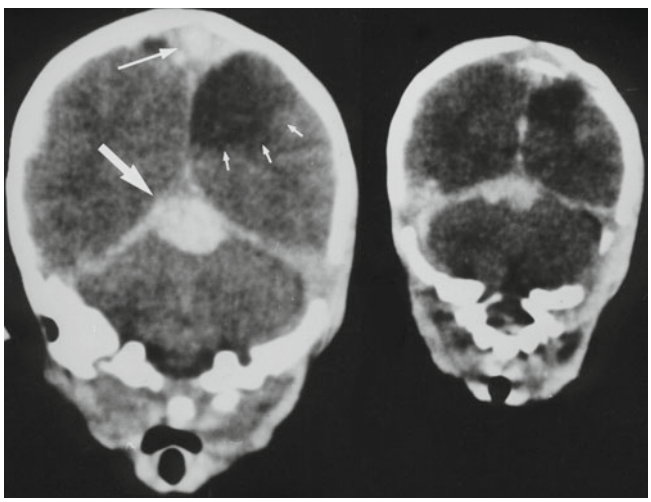

Fig. 142.5 Coronal CT sections in a term infant with sinus thrombosis (*left*) due to impressed occipital squame (*right*). Also observe tentorial subdural hematoma (*large arrow*) and area of ischemia on the left convexity (*small arrows*)

carotid artery or hypoperfusion on that part of the circle of Willis can cause stroke [3]. Chronic cord ischemia has been related to in utero high spinal cord infarction in case of hyperextended breech presentation (Fig. 142.4) [70–72].

CSVT of traumatic origin has been reported as: infected cephalhematoma, osteitis and transverse sinus thrombosis [73], superior sagittal sinus thrombosis due to impression of the occcipital squame [74, 75], propagated thrombosis from superior sagittal sinus into deep veins [5] and transverse sinus thrombosis (Fig. 142.5) [8]. Direct sinus trauma from a penetrating scalp electrode can also induce thrombosis [76].

142.3.4 Step 4: Link Brain Inflammation to Stroke

Both meningitis and ventriculitis can lead to inflammation and occlusion of arteries and veins with focal or diffuse subcortical and/or periventricular infarction [77, 78]. Large vessel NAIS has been reported infrequently, sometimes with exotic organisms: syphilis [79, 80]; *Enterobacter* [81]; *Meningococcus* [82]; *Listeria* [83] (Fig. 142.6); *Pasteurella multocida* [84]; *Salmonella* [85]. Sinus thrombosis similarly can follow bacterial meningitis [86–88]. Local infection may be a portal of entry for thrombophlebitis of a sinus: from scalp infection via emissary veins to a sinus [7, 89], infection in aplasia cutis invading superior sagittal sinus [90, 91] or from orbital cellulitis into the cavernous sinus [92].

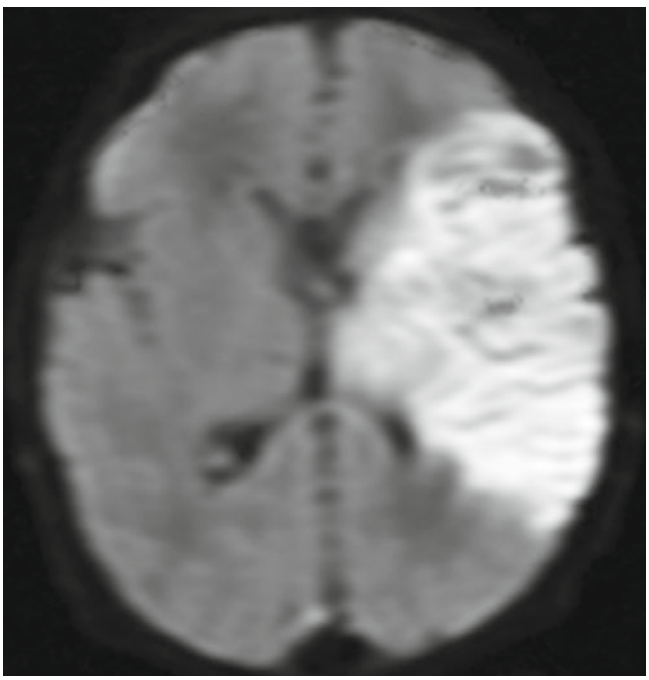

Fig. 142.6 DW MRI: focal large artery stroke (MCA) associated with *Listeria meningitis* in a term infant

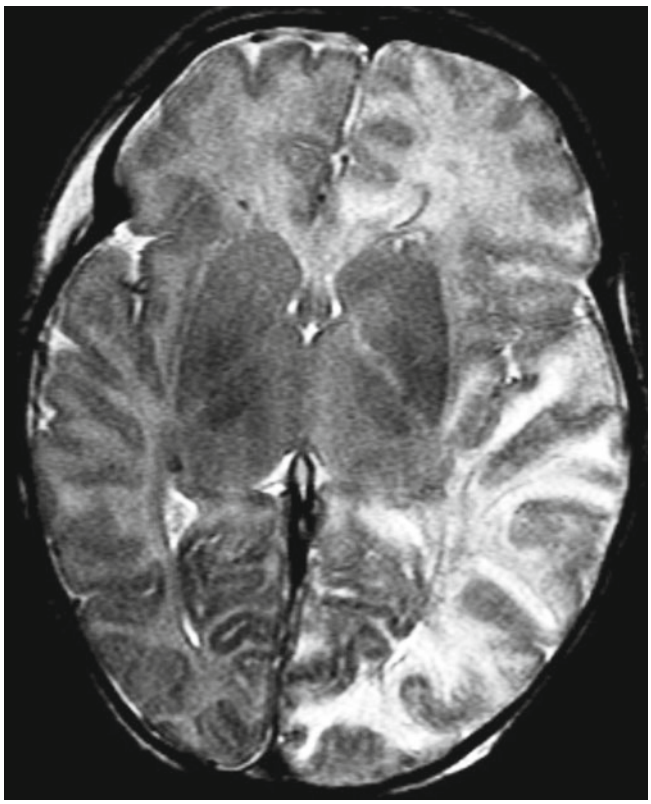

Fig. 142.7 T1 MRI: multifocal ischemic infarction of the left side of the brain in incontinentia pigmenti

Acute chorioamnionitis has recently been suggested as a possible cause of first umbilical venous and later cardiac or brain thromboembolism [93]. There is no evidence for direct cerebral arteritis in this situation. Non-infectious inflammatory conditions can also predispose to arterial stroke. Incontinentia pigmenti probably causes obliteration of small and medium sized arteries in the neonatal period [94] (Fig. 142.7), Aicardi-Goutières syndrome can lead to focal arterial ischemia [95] and infantile arterial calcinosis can induce focal infarction in the basal ganglia and watershed injury at cortical level [96, 97]. Fetal infections like rubella, CMV and toxoplasmosis can be associated with brain arteritis.

142.3.5 Step 5: Recognize an Arteriopathy (Venopathy) or Arterial (Venous) Malformation

Some genetic perinatal arteriopathies were described above with inflammatory conditions. Other hereditary conditions associated with arterial occlusion present in the neonatal period or early infancy: inborn errors of metabolism (hyperhomocysteinaemia, primary hyperoxaluria, galactosialidosis, molybdenum cofactor deficiency, mitochondrial disorders);

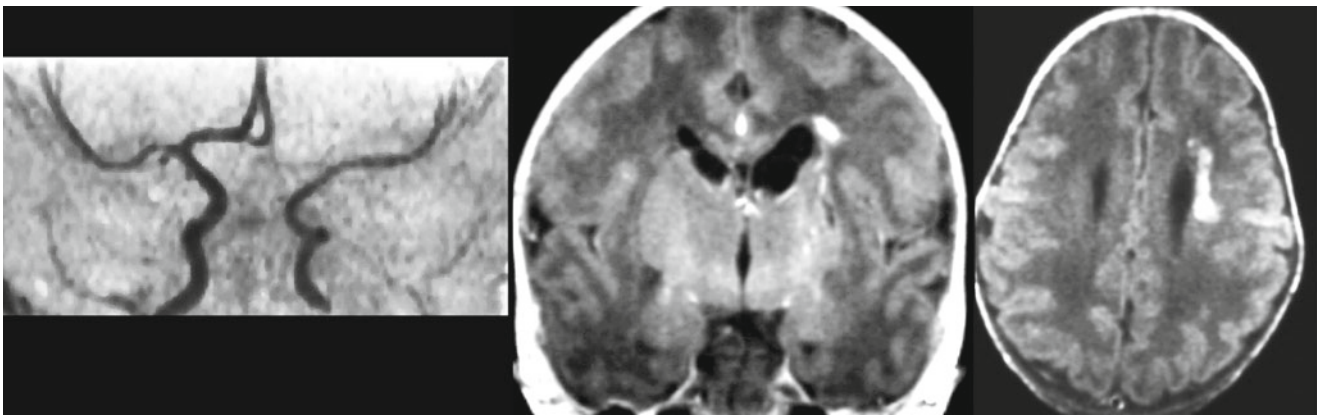

Fig. 142.8 Absence of the left ACA A1 part, attenuated left MCA and ipsilateral periventricular injury, in a term infant with one episode of neonatal convulsions (*left* MRA, *middle* coronal and *right* axial T1 MRI)

anomalies of karyotype (Miller-Dieker lissencephaly, trisomy 13, trisomy 21, 22q11 deletion). Hypoplastic or absent (malformed) arteries can be associated with congenital or postnatal brain tissue loss [98]. Such malformations are seen in PHACE syndrome [99–101] and in Goldenhar syndrome [102]. Carotid hypoplasia may lack an obvious cause [103] or be associated with maternal drug abuse, toxins or infectious agents [26, 104, 105]. Other neonatal arterial anomalies have been linked to fibromuscular dysplasia, to carotid elastin hyperplasia [106], or to Ehlers-Danlos I or Stickler syndrome [107]. We have observed perinatal MCA stroke associated with altered shape and thrombosis of the aortic arch. All these observations warrant documentation of the structure and patency of large intracranial arteries in diverse instances of NAIS (Fig. 142.8). Scarce case reports of sinus thrombosis associated with jugular vein hypoplasia [108] or with abnormal superior vena cava anatomy [109], suggest it may be informative to search for such venous anomalies in instances where neonatal sinus thrombosis is without obvious cause (Fig. 142.8).

142.3.6 Step 6: Find an Embolic Source for Arterial Ischemic Stroke

Emboli find brain arteries in many situations [110]. The source may be: in the left heart (following exchange transfusion, Rashkind procedure, cardiac surgery; due to tumor or arrhythmia); in the carotid or vertebral artery or in the aortic arch; anywhere in the body because of paradoxal embolism through a patent foramen ovale: right heart, umbilical vein, ductus venosus, portal vein, renal vein or inferior caval vein (Fig. 142.9). The existence of a central venous line with thrombus around the tip must be excluded. An embolic mechanism can underly stroke in twin to twin transfusion. Inadvertent injection of embolism-prone material and air embolism are other

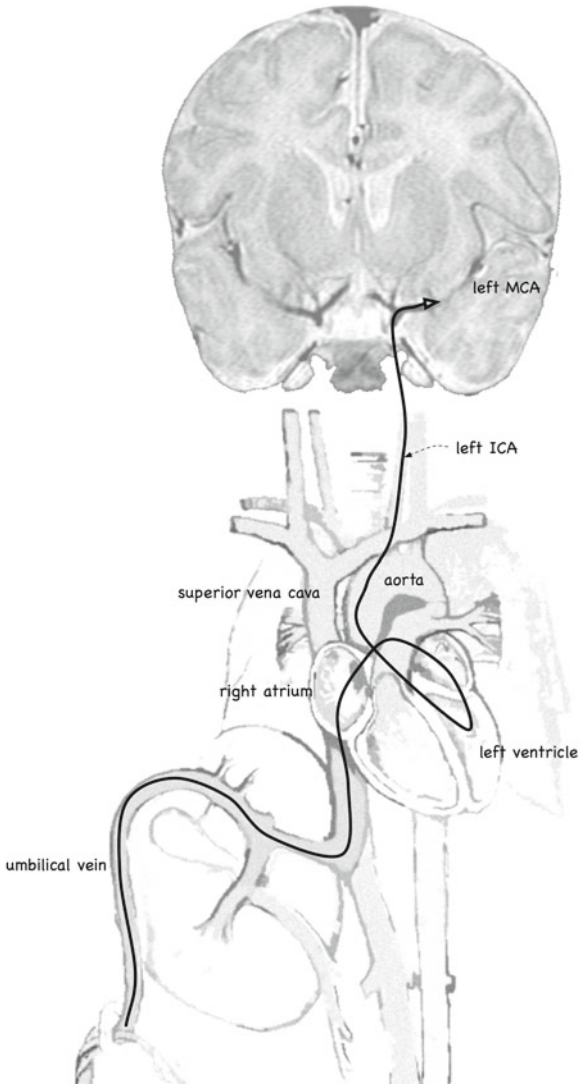

Fig. 142.9 Schematic presentation of paradoxal embolism from inferior caval vein into the left MCA. *ICA* internal carotid artery

possibilities. Flushing a temporal artery catheter [111, 112] and instrumentation of the carotid artery for an ECMO procedure [113] can both cause NAIS. The type of stroke (e.g., the fact that only a trunk of the MCA is affected) may suggest that the mechanism was embolic [114]. It is important to document associated limb (see clinical presentation above) or visceral (heart, gut) infarction: concurrent independent infarction in- and outside the brain increases the likelihood of detecting a prothrombotic condition, an arteriopathy or embolism [115].

142.3.7 Step 7: Diagnose Primary Thrombotic Arterial Ischemic Stroke

Spasm and primary thrombosis are two conditions where vessel occlusion is intrinsic. The balance of hemostasis can be disturbed in the direction of thrombosis, even in the absence of all extrinsic conditions mentioned above. Hypotension (due to infection, heart tamponade or acute blood loss), septicemia and asphyxia probably contribute to thrombosis through endothelial injury [116]. Intrinsic prothrombotic conditions, detailed in the hematological chapters of this book, manifest via several pathways: reduced fibrinolytic activity, enhanced generation or function of thrombin, enhanced platelet aggregation and endothelial dysfunction promoting platelet adhesion.

The extent of testing for genetic prothrombotic risk factors needed in clinical practice is not determined, but extensive screening is often recommended, largely based on experience with older children [117–120]. Prothrombotic risk factors readily find partners in the perinatal period to cause NAIS, as reported in many small cohort and a few case control studies. Familial activated protein C resistance due to factor V Leiden mutation was mentioned first [17, 121, 122]. A modest state of thrombophilia can also be induced by hyperhomocysteinemia above 10 μmol/L. One multicenter case-control study of stroke at term found that none of 91 instances were associated with protein S deficiency [123]. Other factors enhancing the risk of thrombosis detected included increased serum lipoprotein A, homozygous C677T polymorphism in methylenetetrahydrofolate reductase (MTHFR), protein C deficiency and prothrombin (factor II) mutation. Antithrombin deficiency is exceptionally part of the list [124]. Multiple heterozygosity or other risk combinations enhance the prothrombotic tendency. At present there is not enough evidence to prompt clinicians into an exhaustive search for thrombophilia faced with a first and recent perinatal stroke, mainly because of the very low recurrence rate. Doubt remains however because the detection of antithrombin heterozygous deficiency or a combination of genetic prothrombotic risk factors may necessitate secondary prevention with use of anticoagulants during periods of increased risk (immobilization, puberty, pregnancy).

Maternal antiphospholipid antibodies (detected as lupus anticoagulant, anticardiolipin IgG and/or anti-beta2-GPI antibodies in mother and/or infant) may induce thrombosis within the fetoplacental vasculature resulting in paradoxal embolism from the umbilical vein; IgG antibodies may also cross the placenta and potentially cause thrombosis directly within fetal organs [125]. The true significance of fetal thrombotic vasculopathy in relation to NAIS is not yet clear [126]. A hypothetical sequence from amnionitis to fetal vasculitis (and systemic inflammation) to intracardiac thrombosis to embolic stroke was recently discussed [93].

The possible link between arterial ischemic stroke and hyperviscosity (in practice polycythemia) - long ago referred to as a risk factor for lateral striate infarction [127] - has not been recently confirmed. Congenital nephrotic syndrome can present in the neonatal period with both arterial and venous cerebral thrombosis [128, 129]. Congenital disorders of glycosylation (CDG) balance the clotting equilibrium towards thrombosis [130]. Clotting factor suppletion could be a risk factor for NAIS [131].

Prolonged arterial spasm with subsequent infarction, has been linked to endogenous constrictors like subarachnoid hemorrhage, and to exogenous constrictors like cocaine and others (amphetamines, blue cohosh, codein) [132, 133]. Spasm in a brain artery by blunt skull trauma is common in children but has not been reported in the newborn.

142.3.8 Step 8: Diagnose Primary Sinovenous Thrombosis with(out) Infarction

As described above both trauma and inflammation are associated with NCSVT. Venous malformations rarely induce sinus thrombosis. Embolism is not a cause of sinus thrombosis, but the reverse may exist in the newborn: fragmentation of clot in a thrombosed sinus, migration along the jugular vein into the heart and paradoxally into the brain. Hyperviscosity was on occasion mentioned as a risk factor for NCSVT, e.g., in infants of a diabetic mother [134, 135]. The limited number of descriptions of thrombophilia associated with NCSVT does not permit conclusions about the differential sensitivity with arterial thrombosis. Predispositions implicated are: deficiency in antithrombin III, homozygous deficiency of protein C (or S), fV Leiden mutation, fII mutation (Fig. 142.10), the presence of antiphospholipid antibodies, tPAI1 polymorphism, high lipoprotein A, factor VII replacement, congenital nephrotic syndrome and the presence of (post hepatitis B vaccination) antinuclear antibodies [136–147]. The combination of neonatal brain hemorrhage and sinus thrombosis has been recently found with von Willebrand type 3 disease [148]. A contribution of cooling to postasphyxial NCSVT was suggested in a pilot study, but not confirmed later [149]. Dehydration, such as by insufficient breast feeding, is a risk factor specific for venous thrombosis, decades ago referred to as marantic thrombosis [7, 150–152]. Sinus thrombosis can follow endovascular

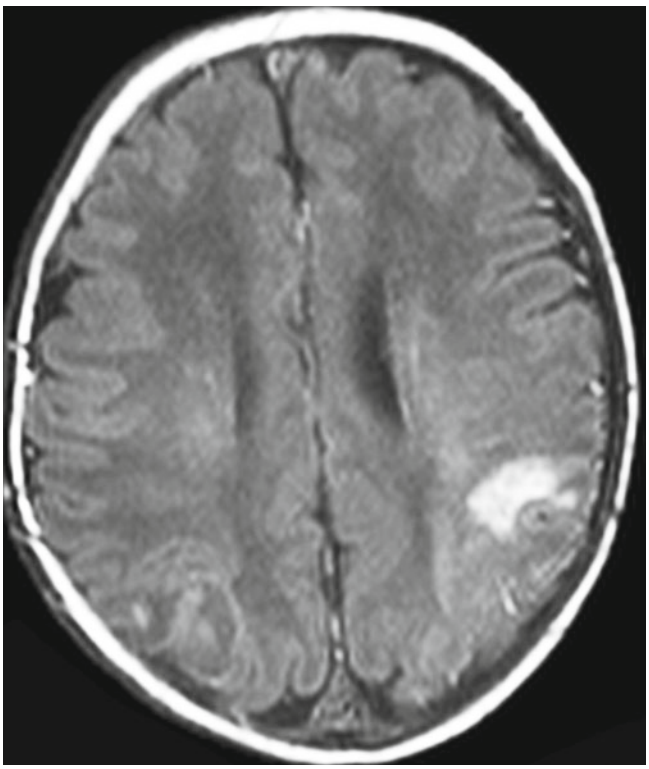

Fig. 142.10 Term infant with first day thrombocytopenia and focal seizures: T1-MR showing superior sagittal sinus thrombosis with hemorrhagic parasagittal infarction

treatment of a vein of Galen malformation [153]. A relation between pre-eclampsia and sinus thrombosis remains to be confirmed [154].

142.4 Medical Treatment

There are guidelines for the treatment of pediatric arterial ischemic childhood stroke: outside pro-active transfusion policy in sickle cell disease, the suggested treatment schedules are mainly based on cohort studies and extrapolation from adult stroke insights (Table 142.1) [155–157]. There are, therefore, no evidence-based treatment options.

In the acute phase of NAIS, supportive measures such as hydration and anticonvulsants are part of standard care. Thrombolysis is not recommended as neither effectiveness nor safety have been demonstrated in neonates. Recommendations propose heparinisation (for a period of months in some cases) in instances with ongoing thrombosis in the heart or a large systemic vein or with carotid artery dissection [158, 159]. For the remainder, anticoagulation or antiplatelet therapy are not recommended as the risk of recurrent stroke is low [160]. A study of 215 neonates with AIS followed for a median of 3.5 years, documented symptomatic recurrence in seven (AIS in four, CVST in two, and deep venous thrombosis in one patient). Factors associated with an increased recurrence rate included thrombophilia and the presence of comorbidities such as complex congenital heart disease or dehydration [125]. Only when AIS reoccurs, therapy is suggested. A heparinization protocol following cardiac catheterization may be useful [161].

It seems obvious that efforts be undertaken to maintain and salvage tissue, particularly within the ischemic penumbra. The synthesis of suggestions for pharmacological intervention from animal stroke experiments is beyond our scope [162]. Easily applicable candidates could be albumin or hypothermia [163]. Insight has to be gained in many areas of NAIS to arrive at treatment options. Theoretically this may facilitate intervention

Table 142.1 Guidelines for treatment of neonatal stroke

		Royal College of Physicians (UK)	American College of Chest Physicians (USA)
Acute AIS	– Acute systemic thrombolysis	No evidence to support use of thrombolytic agents such as tissue plasminogen activator (tPA)	Use of thrombolytic agents in children with AIS has been rare, and the risk/benefit ratio is unknown
	– Acute intraarterial thrombolysis	Not recommended	Not recommended
	– Acute heparinisation	Not recommended, except in case of dissection or cardioembolic stroke	UFHeparin or LMWHeparin for 5–7 days and until cardioembolic stroke and vascular dissection have been excluded
Secondary prevention of AIS	– Dissection	Anticoagulation for ≤ 6 months	Anticoagulation with heparin for 3–6 months
	– Cardioembolic stroke	Anticoagulation in consultation with cardiologist	Anticoagulation with heparin for 3–6 months
	– APL antibodies or inherited thrombophilia	No recommendations	No recommendations
Sinovenous thrombosis		Consider anticoagulation (LMWH or UFH) in selected neonates with – severe thrombophilic disorders – multiple cerebral or systemic emboli – propagating SVT despite supportive therapy Thrombolytic agents are not recommended	Heparin (first unfractionated, later fractionated) is recommended if no significant intracranial hemorrhage is associated, for 6 (if early recanalization is observed) up to 12 weeks (if recanalization is incomplete at 6 weeks)

AIS arterial ischemic stroke, *SVT* sinovenous thrombosis, *LMWH* low molecular weight heparin, *UFH* unfractionated heparin.

at five levels: (i) prevention of risk factors (like vaginal breech delivery or difficult instrumental traction, like catheter-related embolism), (ii) reopening of an occluded vessel with fibrinolytics and/or maintaining collateral patency, (iii) protection of cells in the penumbra from secondary excitotoxic or apoptotic death (by maintaining perfusion and metabolic homeostasis), (iv) prevention of recurrence (in children with important risk factors) and finally (v) specific rehabilitation strategies. Some of these will have to be tested in trials including identical strokes, e.g., complete MCA stroke, to avoid the confounding influence of stroke type on outcome [164–167].

The role of anticoagulants in NCSVT is controversial as recurrency is also rare. Recurrent thrombotic episodes occurred in 6% of children during a mean follow-up period of 36 months [168]. Strikingly, children younger than 2 years of age did not develop recurrent thrombosis at all. However, prolongation of the initial thrombus 1 week after diagnosis has been reported in a cohort study of 68 neonates: in ten out of 40 neonates without anticoagulation (25%), in comparison to one of 28 neonates on anticoagulants (3%). Extension of the thrombus was asymptomatic in all but one neonate, who presented with a novel venous infarction [11]. Although randomized controlled trials were not performed, safety of anticoagulation in NSVT has been monitored [29, 169, 170]. In the Canadian Stroke Registry no patients died or or showed signs of neurologic deterioration due to bleeding during heparin treatment [29]. Consequently, the ACCP guidelines recommend treatment of neonates with SVT without significant intracranial hemorrhage, with unfractionated heparin (UFH) or low molecular weight heparin (LMWH) initially, followed by LMWH or vitamin K antagonists for 6–12 weeks. For neonates with SVT and significant hemorrhage, radiological monitoring is recommended and anticoagulation therapy warranted if extension of the thrombus occurs, 5–7 days after the onset of the initial hemorrhage [156]. Antithrombin concentrates may be needed in addition to heparin in rare instances of sinovenous thrombosis due to antithrombin deficiency and protein C concentrates can be of use in situations where sinovenous thrombosis is associated with protein C deficiency or the presence of antiphospholipid antibodies [125, 171].

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Epidemiology of Adverse Cerebral Outcome

Neil Marlow

143.1 Introduction

Since the beginnings of neonatal intensive care, in particular the recognition that mechanical ventilation could improve survival, there has been debate and discussion as to whether the steady advancement of survival rates comes at the cost of an increased risk of adverse outcomes. This was addressed in 1981 by Stewart, Reynolds and Lipscomb in a review of the world literature on outcomes for very low birth weight (VLBW) births [1]. Since the mid 1940s survival across a range of reports had steadily improved and despite increasing numbers of survivors the proportion of all births surviving with handicaps had remained constant at 6–8%. Indeed, the rate of impaired outcome as a proportion of survivors had progressively increased.

Since then improvements in survival have continued and the lowest birthweight and gestations reported with survival have fallen progressively so that now it is not unusual for babies to survive birth as early as 22 weeks of gestation or weighing < 500 g at birth. These extremely preterm infants (sometimes referred to as previable (not strictly accurate) or micropremies) excite much the same debate as raged in the 1970s over instituting invasive intensive care. It is therefore of great importance to monitor and record the outcomes for such children so that accurate prognostic data can be provided.

One problem is that the numbers of such children cared for by individual institutions over any year is very small – for example in England during 2006 there were only four survivors after birth at 22 weeks and the number of babies surviving at 23 weeks equates to an average of under one per NICU (source EPICure 2). Hence reliable evidence of the proportion surviving with adverse outcome, and with narrow confidence intervals, can only be achieved by averaging over many years, encompassing changing care practices, or by performing large population studies. Population studies are often compared to studies from centers of excellence, something which is really not feasible unless there is 100% referral into such centers, as it is well-described that children survive more frequently in such settings and it is likely that morbidities will be reduced. Thus all reports of comparisons have to come with caveats because of a range of rarely matched issues (Table 143.1). It is possible to make careful comparisons of outcomes between epidemiological studies – for example there are remarkable similarities in outcomes between the EPICure (UK and Ireland) and EPIPAGE (regional study, France) in terms of prevalence of both cerebral palsy and cognitive impairment [2].

Morbidity following extremely preterm birth must be considered with reference to survival as the profit and loss for care at such low gestations is key information for parents. Indeed whereas the outcome for many studies of perinatal care has been traditionally a composite of death of moderate/severe neurodevelopmental impairment, increasingly unimpaired outcome is being preferred as that is the goal of our care [3]. However, for parents the guide still remains the risk of death or serious disability.

Since the mid-1990s there have been several population based studies of outcomes among extremely preterm children (Fig. 143.1). They report a range of outcomes of which two predominate – cerebral palsy and serious developmental delay. Such studies cannot be directly compared, however, because of differences in population definitions of extremely preterm birth (some include births 22–25 weeks and others up to 27 weeks), differences in definitions of disability, variation in follow-up rate, different methods of obtaining denominator data (for survival) and differences in baseline prematurity rates in different populations (Table 143.1).

143.2 Cerebral Palsy

The prevalence of cerebral palsy (CP) increases with both decreasing gestational age and birthweight. The rate of CP in

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Table 143.1 Pitfalls in the comparison of data from different studies of adverse outcomes

Problem		Suggested solution for reports
Gestational ages and birth weights	*No agreed definition of "extremely low GA", more consensus over birthweight categories*	Report in fixed categories by: – single completed gestational weeks (to 30 w) – 250 g weight intervals (to 1500 g) – always use confidence intervals
Population bases	*Some report hospital based outcomes other community or population*	Population data preferred for comparative data as circumvents issues of referral bias
Perinatal baseline	*Some units report outcomes only for admissions, others live births, others for babies alive at the onset of labor*	Report all three statistics if possible; important as parents need updated risk information depending upon their pathway though labor and delivery
Assessment ages	*Most authorities agree that more accurate data come from later acquisition of information*	18–24 months is the most common age of initial assessment and should be adhered to
Outcome measures	*Variation in definition, cut offs, and tests used*	Use standard published definitions of outcome and ensure contemporary developmental tests, where not possible collect reference or comparator data for term infants
Population	*Immigration and social disadvantage important contributors to rate of preterm birth and morbidity*	Evaluate and report population mix in terms of ethnicity, social advantage and baseline prematurity rate
Health systems	*No clarity on policy al extremely low GA in terms of options for parents and for babies*	State policy (even if cannot be agreed, estimate variation) and proportion of children at each GA week in whom resuscitation is attempted

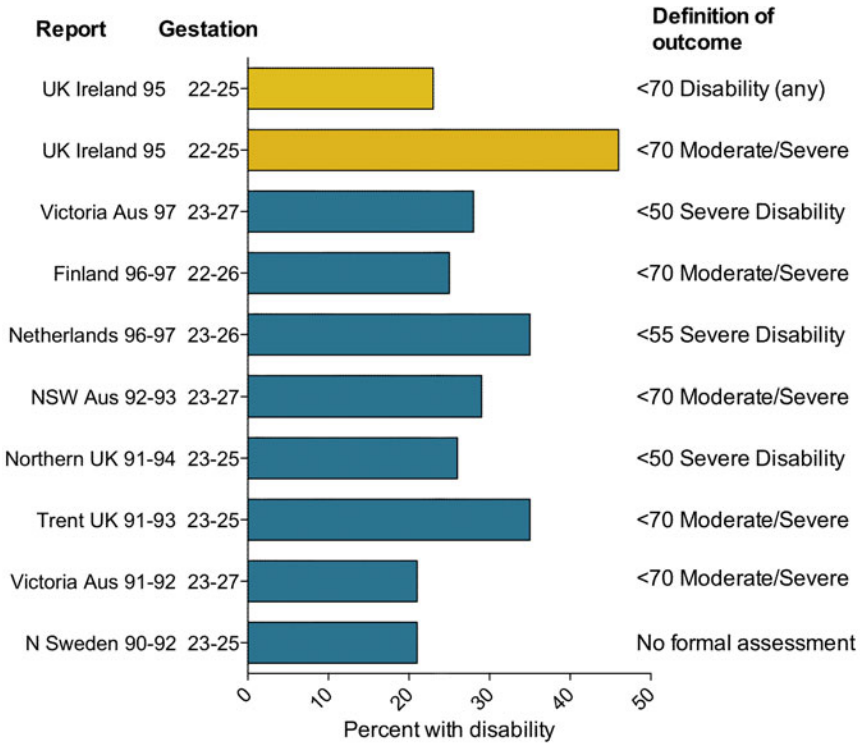

Fig. 143.1 Summary of outcomes from national or geographically based studies of outcome in extremely preterm infants showing the variability in the gestation range included and in the definition of outcome. Two definitions are shown for the UK and Ireland report to demonstrate the effect of differences in definitions. Data adapted from [20]

the individual populations cited in Fig. 143.1 varies between 10 and 20% with all the caveats mentioned above. Better data come from the reports of population studies, such of those of Hagberg and colleagues from southern Sweden, (Fig. 143.2) [4] or the *UK 4*-Child register. In these studies care has been taken to use a geographically based approach and careful case definition to produce consistent trends.

The rise in prevalence of all types of CP seen from the late 1960s has now been replaced by a steady decline during the 1990s [4]. In particular, there has been a decrease in the

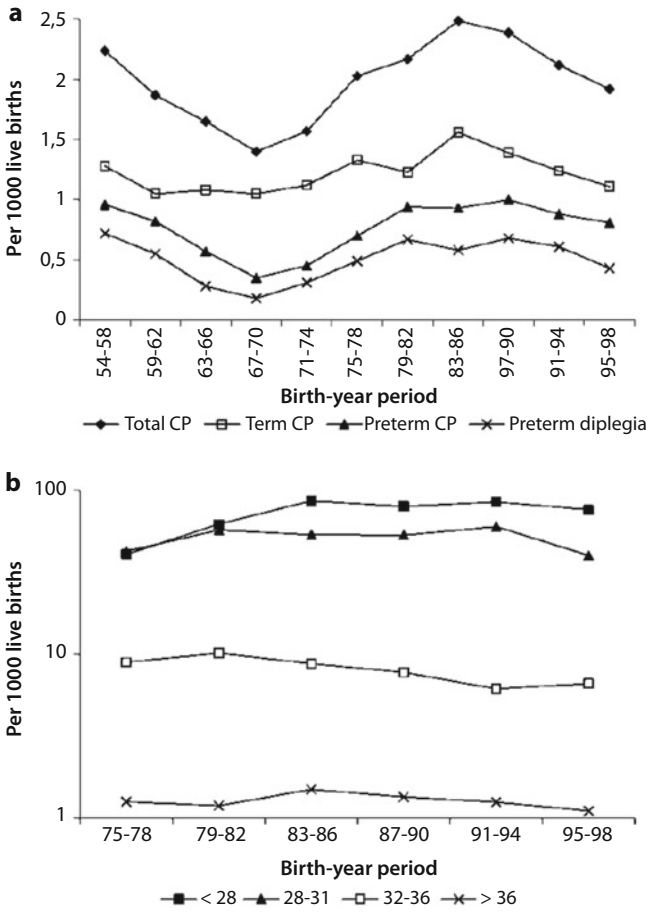

Fig. 143.2 Trends in the prevalence of cerebral palsy in Southern Sweden 1954–1998. **a** Prevalence by type of cerebral palsy; **b** Gestation specific prevalence. Reproduced from [4], with permission

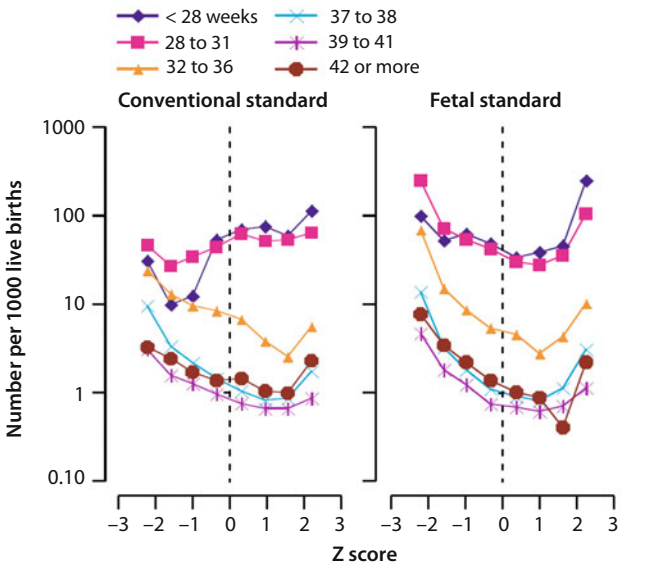

Fig. 143.3 Prevalence of cerebral palsy by gestational age and size at birth: effect of different growth standards. Reproduced from [9], with permission

prevalence of diplegia, itself associated with preterm birth, and a small increase in dyskinetic types of CP – more associated with term acute intrapartum hypoxia. Furthermore CP associated with preterm or very low birthweight births tends to have less associated disability [5, 6].

It seems clear that the population risk of CP is relatively stable or falling slightly but that, as the children born at extremely low gestational age (GA) (in whom survival is increasing) predominate, in individual gestational age categories above 28 weeks it seems likely that the prevalence is falling in keeping with the reduction seen in ultrasound diagnosed brain-injury from hemorrhagic parenchymal infarction and cystic periventricular leukomalacia (PVL). This mirrors the observation of Pharoah and colleagues that birthweight specific prevalence of CP first increased in line with survival (a possible practice effect) before falling as practice improves further [7]. CP is also more common among children who are small-for-gestational age at birth. For some time it has been considered that the effect of prematurity swamped any relationship at gestational ages below 34 weeks [8]. In fact most preterm children are in fact small at birth compared to well fetuses of a similar gestational age. When estimated fetal weight standards are used this relationship is clearly seen (Fig. 143.3) [9]. Interestingly, it is seen that being large-for-gestational age also increases risk across the gestational range.

143.3 Developmental or Cognitive Outcomes

There are fewer systematic studies associating cognitive outcomes over time. Partly this is due to the difficulty of drifting test norms and evolving developmental or psychometric test development, making it very difficult to compare changes over time.

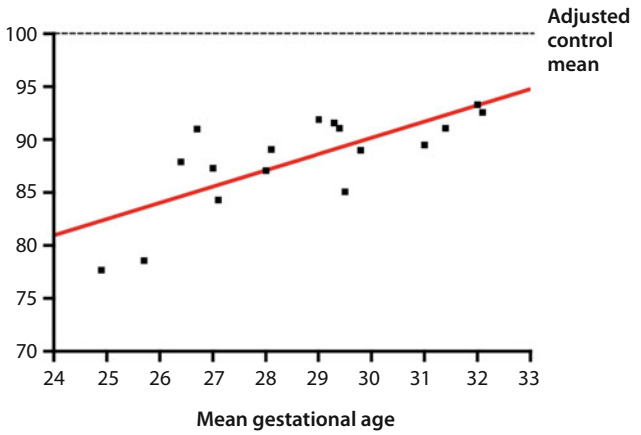

Fig. 143.4 Relationship between mean IQ and mean GA in 17 studies reporting outcomes with comparison populations; scores have been normalised to a consistent comparison population score of 100. Reproduced from [13], with permission

Cognitive scores are related to gestational age at birth. In a whole population study in Bavaria a clear relationship is seen with no significant correlation at gestations above 33 weeks whereas below 33 weeks there is a steady fall off with gestational age [10]. The EPICure data are consistent with this trend [11] and if one evaluates the mean IQ of studies of very preterm children by their mean GA at birth there is a similar relationship (Fig. 143.4) [12, 13].

The relationship with size at birth in epidemiological studies is less clear. Where there is evidence of fetal growth restriction, cognitive scores are reduced [14, 15] but recent data suggest this is not so [16]. Systematic reviews indicate that an effect by gestational age is difficult to determine [17, 18], but in some recent large studies scores tend to be lower than appropriately grown babies. Some of this variation must relate to policy of delivery early or later in the course of evolving growth restriction, although evidence for this is wanting [19].

Further discussion of these areas is outside the remit of this chapter but may be found in Chapter 129.

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144

Neuromuscular Disorders

Paolo Balestri and Salvatore Grosso

144.1 Introduction

Neuromuscular disorders (NDs) are important causes of neonatal weakness and hypotonia. They are caused by disorders affecting the motor unit which anatomically includes a motor neuron, its axon, the neuromuscular junction, and all muscle fibers innervated by that single motor neuron [1].

NDs can be acquired or genetically determined with variable inheritance [2, 3]. A transient neuromuscular disorder such as neonatal myasthenia may affect children born to mothers with myasthenia gravis as a consequence of passive transfer of the antibody across the placenta [2].

Chief laboratory investigations include:

- Serum creatine kinase (CK) levels which, although a highly sensitive indicator of a muscle disorder, can be normal in some congenital dystrophies and congenital myopathies. High CK levels, up to 1000 units (normal adult level < 200 IU/L), are commonly observed during the first few days of life after vaginal delivery, reflecting muscle damage during labor;
- Motor nerve conduction studies which may detect a disorder of the peripheral nerve;
- Electromyography (EMG), which provides information about every level of motor unit, is generally obtained with the muscle at rest and with spontaneous or elicited contractions, but may be difficult to interpret as severely hypotonic neonates make few spontaneous movements;
- Muscle biopsy is often a crucial diagnostic procedure in the evaluation of a child with a disorder of the motor unit. In neonates, indications for muscle biopsy should be carefully considered in patients in whom the diagnosis remains uncertain in spite of laboratory, electrophysiologic and DNA analysis investigations [2, 3].

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144.2 Congenital Myopathies

The congenital myopathies (CM) are a group of disorders characterized by: (1) distinctive and specific morphologic anomalies in skeletal muscle on light and/or electron microscopy as the main pathological feature (i.e., cores, rods, and central nuclei); (2) pathological features originating within the myofiber and, therefore, distinct from the muscular dystrophies where the primary pathology affects the stability of the sarcolemmal membrane; (3) a genetic etiology; and (4) clinical presentation with weakness and hypotonia. A neonatal onset with delay in motor milestones can be observed [4].

144.2.1 Central Core Disease

144.2.1.1 Etiology and Pathogenesis

Central core disease (CCD) is an autosomal dominant disorder related to mutations in the RYR1 gene which encodes for the skeletal muscle ryanodine receptor involved in the internally stored calcium and in the excitation-contraction mechanisms [5]. Pathological features include the presence of cores (unstained) on oxidative stains in the sarcomeres of type 1 fibers, which are central or somewhat eccentric in location. The exact mechanism of core formation is not clear. They are immunohistochemically characterized by increased RYR-1 protein, desmin, vimentin, tubulin and developmental myosin heavy chain [6].

144.2.1.2 Clinical Aspects

CCD shows considerable clinical variability and severity. Cardinal features are early onset hypotonia, weakness mainly affecting the proximal muscles, and hip dislocation. Patients with a static or nonprogressive course may achieve motor

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

milestones, although at a slow rate. Contractures and kyphoscoliosis may occur with progressive weakness. Extraocular muscle weakness, bulbar signs and respiratory impairment do not occur [6–8].

144.2.1.3 Laboratory Studies

CK levels are usually normal and EMG shows evidence of a mild myopathy. Muscle biopsy is crucial for the diagnosis. Muscle magnetic resonance (MR) imaging has a role in the diagnosis of CCD [9]. Patients with RYR1 mutations have a recognizable pattern of muscle involvement irrespective of the variability of histopathological features [7, 8].

144.2.1.4 Differential Diagnosis

During the neonatal period, classical histopathological abnormalities may be lacking and the differential diagnosis from multi-minicore disease may be difficult. Peculiar clinical features such as external ophthalmoplegia and respiratory impairment, commonly lacking in CCD, may help in differentiation [6, 8]. Nemalinic myopathy (NM) with mutations in the *ACTA1* gene has also been associated with both central core and minicores [10].

144.2.2 Nemaline Myopathies (NM)

144.2.2.1 Etiology and Pathogenesis

NM is genetically heterogeneous: both autosomal dominant and recessive models of inheritance have been observed. Mutations in five different genes have been detected. ACTA1 gene changes account for the 60–75% of cases with severe congenital nemaline myopathy and are related to the aggregation of actin filaments and the presence of rods within the nuclei of muscle fibers (the so-called intranuclear rod myopathy) [4–6, 11, 12].

144.2.2.2 Clinical Aspects

Six different clinical forms of NM have been described. Three present during the neonatal period. They include:

1. Typical congenital NM, which presents with hypotonia and weakness.
2. Severe congenital NM, which is characterized by lack of muscular movements, respiratory insufficiency, severe contractures, arthrogryposis multiplex and, sometimes fractures. Polyhydramnios, decreased fetal movements, and abnormal fetal presentation or fetal distress can also occur.
3. Intermediate congenital NM, which is a variant in which, as in the severe form, neonatal hypotonia and weakness are present but the child is able to perform spontaneous movements and no severe contractures or respiratory insufficiency are observed. Facial weakness occurs in almost all patients. Ophthalmoplegia is lacking [3, 13, 14].

The severe and the intermediate forms of NM have a poor prognosis. Mortality within the first year of life is directly related to respiratory insufficiency at delivery and the presence of arthrogryposis multiplex congenital. A stormy early course characterized by severe weakness and impaired feeding at birth with the need of mechanical ventilation and frequent respiratory tract infections may be followed by clinical stabilization and, in some, ability to stand or walk [3, 13, 14].

144.2.2.3 Laboratory Studies and Pathology

CK serum levels are either normal or mildly increased. EMG shows evidence of a myopathy. Muscle biopsy shows threads-like inclusions on modified Gomori trichrome (MGT) staining (Fig. 144.1). There is predominance of type I fibers. Ultrastructural studies show rod bodies, which are derived from the lateral expansion of the Z-band and mainly contain alpha-actinin and tropomyosin [3, 14–16].

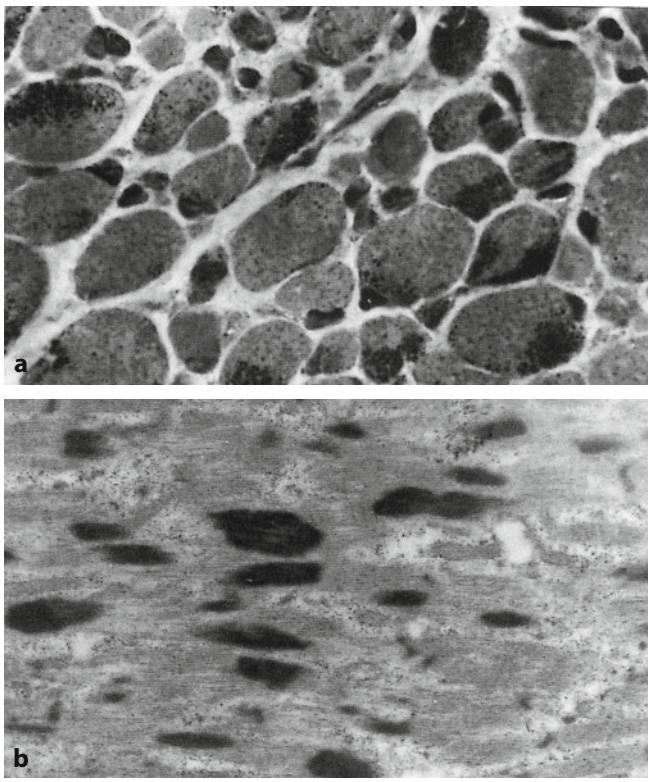

Fig. 144.1 **a** Gomori's trichrome reaction, transverse section (900×). Presence of numerous typical rod-shaped bodies. **b** Longitudinal view of a fiber with several nemaline bodies measuring 3–5 μm in length and 1–2 μm in width (14,000×). Reproduced from [15] with permission

144.2.2.4 Treatment

Therapy includes aggressive management of pulmonary infections and feeding difficulties.

144.2.3 Myotubular Myopathy

Autosomal dominant, autosomal recessive and X-linked recessive forms of myotubular myopathy (MM) have been recognized. The term MM, which refers to muscle fiber resembling fetal myotubules, continues to be used to indicate the X-linked variant with neonatal onset [3, 5, 17].

144.2.3.1 Etiology and Pathogenesis

Neonatal MM is caused by mutations in the XLMTM gene located at Xq28, encoding a protein, called myotubularin, which belongs to the family of tyrosine phosphatase. The reasons why this enzyme defect induces muscle fiber immaturity remain to be elucidated.

144.2.3.2 Clinical Aspects

Neonatal MM has a severe phenotype. Males present at birth with marked weakness and hypotonia, external ophthalmoplegia and respiratory failure [17]. The majority of patients die within the first months of life. A positive family history of either male neonatal deaths or miscarriages is common. Reduced fetal movements and polyhydramnios are common. Marked facial weakness, ptosis, ophthalmoplegia, joint contractures, high-arched palate, club feet, micrognathia, and arthrogryposis multiplex are also present, as well as macrosomia and cryptorchidism. Thinning of the ribs on chest radiographs, rarely observed in other congenital myopathies, has been reported.

Birth asphyxia, often misinterpreted as an hypoxic-ischemic encephalopathy, may be the presenting feature. Only a proportion of affected males survive into their teens or beyond. In these cases, motor outcome is poor as many of them are bedridden or wheelchair-bound [18].

144.2.3.3 Laboratory Study and Pathology

CK serum levels are either normal or mildly increased. EMG shows evidence of a myopathy. Muscle pathology shows fibers containing central nuclei. Immunohistochemical studies demonstrate increased signal from myofibers with antibodies to proteins such as vimentin, desmin, neural cell adhesion molecule, and utrophin, probably related to the immaturity of myofibers [18, 19].

144.2.4 Congenital Fiber Type Disproportion

Congenital fiber type disproportion (CFTD) is a rare type of congenital myopathy which may be sporadic or familial. Mutation in genes such as ACTA1, SEPN1, and TPM3 have been detected.

144.2.4.1 Clinical Aspects

CFTD is characterized by hypotonia and mild-to-severe generalized muscle weakness of limbs, neck, and face. The onset is at birth or within the first year of life. There are also bulbar signs, extraocular muscle involvement and respiratory failure, which can occur at any age. Neonatal disability may be severe with contractures of the hips, knees, ankles, elbows, and fingers.

The course of the disorder is variable and some individuals may be able to walk.

144.2.4.2 Laboratory Study and Pathology

CK serum levels are mildly increased. Histopathology shows type 1 fiber predominance and hypoplasia. Fiber type disproportion can also occur in neurogenic atrophy and in many other muscle disorders. Its existence as an independent entity has therefore been questioned [3–6, 19].

144.2.5 Multi-Minicore Disease

Multi-minicore disease (MMD) has a wide clinical spectrum with four distinct phenotypes.

144.2.5.1 Etiology and Pathogenesis

Sporadic and autosomal recessive cases have been linked to mutations in both SEPN1 and RYR1 genes. Multiple minicores, small zones of sarcomeric disorganization lacking mitochondria in muscle fibers, affect both type I and type II fibers.

144.2.5.2 Clinical Aspects

The onset of classical MMD is during the neonatal period or within the first year of life with a peculiarly slow course. Clinical features are hypotonia, generalized weakness mostly affecting proximal muscles, spinal rigidity, scoliosis, and respiratory impairment.

Arthrogryposis can be observed in MMD patients carrying RYR1 mutations (3-6, 19).

144.3 Congenital Muscular Dystrophies

Congenital muscular dystrophies (CMD) are a group of genetically and clinically heterogeneous hereditary myopathies with a predominantly autosomal recessive model of inheritance. Striking myopathological findings include great variability in the size of muscle fibers, marked endomysial and perimysial proliferation, and later increase of adipose tissue. Internal nuclei and necrotic as well as regenerating fibers can be observed during the early stages. The clinical course is widely variable. CMD patients may have central nervous system (CNS) and ocular changes and may not exhibit a progressive course.

A classification of CMDs based on biochemical and genetics defects has been proposed and continuously up-dated. Most of the various genes involved in the pathogenesis of the CMDs are related to the function of the dystrophin-glycoproteins associated complex (DAG) in the sarcolemma and may lead to abnormalities of either extracellular matrix proteins or glycosylation of alpha-DG. A single gene may be related to diverse clinical phenotypes. For the clinician, it may be more practical to define CMDs according to the involvement of the CNS and the presence or absence of merosin (i.e., merosin-positive and merosine-negative CMD) [19–22].

144.3.1 Merosin-Negative CMD

144.3.1.1 Etiology and Pathogenesis

Merosin is the critical alpha-dystroglycan-binding, basement membrane protein of the muscle extracellular matrix. Merosin defects are linked to LAMA2 gene mutations. White matter anomalies are consistently present in merosin-genative CMD.

144.3.1.2 Clinical Aspects

CMD caused by a primary deficiency of merosin accounts for up to 50% of CMD and differs from the classic merosin-positive CMD.

The clinical picture of CMD with total absence of merosin expression is homogeneous: there is a severe phenotype characterized by marked muscle weakness, atrophy, and facial weakness with impaired chewing and swallowing. Diffuse joint contractures are common. Patients are unable to achieve independent ambulation. In one third, death supervenes during the first year of life because of cardiopulmonary complications. Serum CK levels are markedly raised and white matter changes represent a consistent finding on brain MR imaging.

Cognitive impairment, seizures, and neuronal migration defects, mainly in the occipital cortex, have been reported in a few patients [3, 19–21].

144.3.1.3 Investigations and Pathology

Serum CK levels are markedly elevated. EMG shows evidence of a myopathy. Muscle biopsy demonstrates a classic dystrophic process with fiber necrosis and regeneration [19–22].

144.3.2 Congenital Muscular Dystrophies with Structural Anomalies of the CNS

These dystrophies are mainly represented by Fukuyama congenital muscular dystrophy (FCMD), Muscle-Eye-Brain (MEB) disease, and Walker-Warburg syndrome (WWS). Ocular anomalies and severe brain changes such as disorders of neuronal migration, cerebral white matter abnormalities, and hindbrain anomalies can be present. The pathogenesis of CMD is through abnormalities of glycosylation of alpha-dystroglycan (alpha-DG) and therefore grouped under the name of dystroglycanopathies.

144.3.2.1 Etiology and Pathogenesis

They show an autosomal recessive inheritance with mutations in at least six genes (POMT1, POMT2, POMGnT, fukutin, FKRP, and LARGE), which encode specific or putative glycosyltransferases involved in the glycosylation of alpha-DG. It was earlier suggested that each CMD form was caused by mutations in a distinct gene. It is now clear that WWS and MEB are linked to mutations in all the genes involved in the glycosylation of alpha-DG. Conversely, mutations in one gene may give origin to variable clinical features, with diverse CNS and ocular patterns of anomalies.

144.3.2.2 Investigations and Pathology

CK serum levels are increased. Normal CK values do not exclude the diagnosis. EMG may show evidence of a myopathy sign. Muscle biopsy changes, compatible with a dystrophic process, may also appear late during the clinical course. Brain imaging and ophthalmologic examination are necessary to define CNS and ocular anomalies [3, 19–23].

144.3.2.3 Clinical Aspects

Fukuyama Congenital Muscular Dystrophy (FCMD)

FCMD mostly occurs in Japan. There is variable severity even in the same family. Clinical features include poor fetal movements, impaired respiration, generalized weakness, hypotonia, and facial weakness. Joint contractures, hypertrophy of the calves, quadriceps and tongue muscles; a cardiomyopathy

may develop later. Mental retardation and seizures are cardinal features. CNS anomalies are represented by cerebral and cerebellar micropolygyria, obstructive hydrocephalus, occipital cobblestone cortex, hypoplasia of the pons and cerebellar vermis, and cerebellar cysts. Ocular changes include retina anomalies with folding, fusion, focal dysplasia, and detachment, abnormal eye movements, strabismus, myopia and microphthalmos [3, 19, 21, 24].

Walker-Warburg Syndrome

This is the most severe of CMDs. Neonatal generalized weakness and severe hypotonia are associated with brain malformations (lissencephaly type II, prenatal obstructive hydrocephalus, neuronal heterotopias, corpus callosum agenesis, pontocerebellar hypoplasia, and sporadically occipital encephalocele and Dandy-Walker cyst). Ocular changes involve both anterior and posterior eye chambers (retinal detachment, blindness, microphthalmia, optic nerves hyploplasia, colobomas, congenital glaucoma, cataract, megalocornea). Genital anomalies in males, and facial dysmorphisms with cleft lip or palate have been reported [3, 19–21, 25].

Muscle-Eye-Brain Disease (MEB)

Initially reported in Finland, MEB is now recognized worldwide. Although it is less severe than WWS, the spectrum of clinical severity is broad with patients presenting a WWS-like phenotype. Ocular defects include high myopia, congenital glaucoma, retinal dysplasia, coloboma of the optic nerve, and cataracts. CNS abnormalities are represented by cortical malformations variable in severity within the spectrum of pachygyria/polymicrogyria/agyria associated to flat brainstem and cerebellar hypoplasia, and white matter abnormality. Hydrocephalus, seizures and facial dysmorphisms (short nasal bridge, micrognathia, and midface hypoplasia) may also be observed. The clinical course is variable with some patients able to walk and speech, while others present with severe motor and cognitive delay [21, 22].

144.3.3 Congenital Myotonic Dystrophy

In the congenital form of myotonic dystrophy muscle weakness predominates and may be so severe as to lead to neonatal death [19].

144.3.3.1 Etiology and Pathogenesis

Congenital myotonic dystrophy is caused by mutations in the DMPK gene which contains an unstable CTG trinucleotide. CTG repeats range from 5 to 34 in normal people. These patients have very large CTG repeats, which number up to several thousands. The mother is almost always the parent who transmits the larger repeat, but transmission by the father has been recorded [26].

144.3.3.2 Clinical Aspects

The clinical picture include polyhydramnios, reduced fetal movements, severe generalized weakness, hypotonia, arthrogryposis (especially of the lower extremities), feeding difficulties, and respiratory insufficiency with mechanical ventilatory support needed for most of affected children. Facial diplegia, with a classical inverted V-shaped upper lip, is constant. Areflexia and atrophy are common. In neonates, percussion myotonia, where percussion of a muscle evokes a mytonic phenomenon, is elicited only sporadically. Mortality from respiratory failure is high [19, 21].

Severity of the clinical course is consistent with the clinical picture at onset. Neonatal mortality can be up to 40% in severely affected children. In the post-neonatal period most patients survive but few become independent [27]. A careful examination of the mother may show a typical myotonic facies with ptosis, atrophy of the masseter and temporalis muscles, and percussion myotonia. Presenile cataract, frontal baldness, and gonadal atrophy may also be seen in affected family members.

144.3.3.3 Laboratory and Pathology

Serum CK level is normal. EMG leads to the diagnosis with myotonic discharges. Muscle pathology features are not diagnostic [3].

144.4 Metabolic Myopathies

Disorders with prominent involvement of muscle at neonatal onset mainly include defects of glycogen metabolism and respiratory chain.

144.4.1 Pompe Disease (PD)

PD is a lysosomal storage disorder that leads to accumulation of glycogen with muscle weakness, organ damage and death. It is detectable by screening during the newborn period, and treatment has become available. The clinical picture is characterized by early onset severe weakness and hypotonia, mimicking SMA1. The diagnosis is suggested by the observation of cardiomyopthy, macroglossia and hepatomegaly, which are lacking in SMA1 [2, 3]. Enzyme replacement therapy with recombinant human precursor acid α-glucosidase is available.

144.4.2 Mytochondrial Myopathies (MM)

MM are highly heterogeneous conditions related to mitochondrial DNA or nuclear DNA mutations with defects of oxidative phosphorylation. They are generally multi-system and affect highly aerobic post-mitotic tissues, such as muscle and nerve. Autosomal recessive inherited defects of cytocrome C oxidase may present with a neonatal myopathy syndrome. Cardinal clinical features are axial and facial weakness, hypotonia, hypo/areflexia. Systemic involvement is also common with hepatomegaly, cardiomyopathy and renal dysfunctions. Lactic acidosis and the presence on muscle biopsy of ragged red fibers with cytocrome C oxidase deficiency are diagnostic [3].

144.5 Myasthenia

144.5.1 Neonatal Mysthenia Gravis

Neonatal myasthenia gravis (NMG) is a transient disorder of neuromuscular transmission that occurs in 12% of newborns born to women with myasthenia gravis [28].

144.5.1.1 Etiology and Pathogenesis

NMG is related to the passive transfer of antibodies against acetyl-choline receptor (AChR) across the placenta, which it is thought to be followed by decreasing AChR at the postsynaptic muscle membrane.

144.5.1.2 Clinical Aspects

Clinical onset usually occurs within a few hours of birth or during the first 72 hours. There may be facial diplegia with feeding, sucking and swallowing difficulties, generalized weakness, hypotonia, respiratory insufficiency and inability to handle pharyngeal secretions. Ptosis and oculomotor dysfunctions occur in a minority of patients. The most severely affected infants may show arthrogryphosis, polyhydramnios, pulmonary hypoplasia and death.

144.5.1.3 Investigations

Repetitive nerve stimulation, at 5-20 Hz, typically shows a decrement in the amplitude of the motor action potential.

144.5.1.4 Treatment

Neostigmine, an anticholinesterase, is the drug of choice. Initial doses of 0.04 mg/kg intramuscularly, are followed by 0.4 mg/kg given by gastric tube. Exchange transfusion is ineffective. Few data are available on the efficacy of intravenous immunoglobulins [2, 19].

144.5.2 Non-Autoimmune Congenital Myasthenic Syndromes (CMSs)

These syndromes are inherited heterogeneous disorders affecting the neuromuscular junction. In most patients clinical onset is during infancy. The number of recognized CMSs has increased dramatically over the past few decades. They are classified by the site of neuromuscular transmission defect (presynaptic, synaptic or postsynaptic), but accurate identification of the site of the defect is not always possible.

144.5.2.1 Etiology and Pathogenesis

In some patients a deficiency of endplate acetyl-choline receptor (AChR), and referred to as "Endplate Acetylcholine Receptor Deficiency", can be identified. Autosomal recessive inherited mutations in genes coding for both AChR subunits and for rapsyn, a protein able to cluster the receptors, have been reported. CMSs with presynaptic dysfunctions, referred to as congenital myasthenic syndrome with apnea are linked to a gene encoding choline acetyltransferase, a rate-limiting enzyme involved in resynthesis of acetylcholine.

144.5.2.2 Clinical Aspects

Although phenotypic variability exists among CMSs, several clinical features are shared by these disorders. Ptosis, ophthalmoplegia, early facial and bulbar involvement with feeding difficulties and respiratory failure, hypotonia, and weakness are common. Reduced fetal movements, arthrogryposis and polyhydramnios may be observed. Peculiar clinical findings may occur in specific CMS syndromes (i.e., pupillary dysfunction is typical of endplate AChE deficiency).

144.5.2.3 Investigations

Investigations include single fiber-EMG, specific staining for AChR, electron microscopy of motor endplate, and molecular genetic studies.

144.5.2.4 Treatment

Anticolinesterase drugs are crucial in the therapeutic strategy. 3,4-Diaminopyridine is effective in combination with anticolinesterase. Steroids and thymectomy are ineffective. Medication should be protracted: an interruption may lead to apnea and sudden death [2, 19, 28–31].

144.6 Spinal Muscular Atrophy (SMA)

The spinal muscular atrophies (SMAs) comprise a group of autosomal-recessive disorders characterized by progressive weakness linked to loss of the lower motor neuron in the anterior horn cells in the spinal cord and cranial nerve nuclei. Several types of SMAs have been defined by their age at onset. A neonatal onset occurs in the acute, severe infantile variant (SMA type 1; Werdnig-Hoffman disease) [32].

144.6.1 Etiology and Pathogenesis

The gene for the autosomal recessive SMA1 is located on 5qll-ql3 and termed survival motor neuron (SMN). Each individual has two SMN genes, SMN1 and SMN2. More than 95% of patients with SMA1 have a homozygous mutation in the SMN1 gene [33].

144.6.2 Clinical Aspects

SMA1 present with axial and limb weakness and hypotonia at birth or in the first weeks of life. Legs are more affected than arms and proximal muscles more than distal. There is little spontaneous activity, which is limited to the feet and hands. Facial muscles are spared and the infants show a normal bright expression. Bulbar dysfunction includes an impaired ability to suck, reduced swallowing, and respiratory failure. The intercostal muscles are always affected but the diaphragm is spared. The extraocular muscles are not involved and there is normal brain anatomy. Almost all patients die from complications of the disease within 18 months. At physical examination the extensive paralysis of the limbs and distinctive breathing pattern are associated with the characteristic jug-handle posturing of the upper limbs with internal rotation of the shoulders and pronation of the forearms. Deep tendon reflexes are absent and fasciculations are often restricted to the tongue. Findings on sensory examination are normal. Arthrogryposis or deformities of the limbs and joints at birth can be observed as a result of utero hypotonia [2, 32, 34].

144.6.3 Investigations

Serum CK levels are normal. Electrophysiologic studies help to differentiate SMA1 from other neurogenic and myopathic diseases. The SMN1 gene should be screened for mutation. Muscle biopsy should be considered in the diagnostic workup when genetic analysis is normal.

144.6.4 Spinal Muscular Atrophy Variants

Among variants of SMA, those with a potential neonatal onset include the spinal muscular atrophy with respiratory distress type 1 (SMARD1) and pontocerebellar hypoplasia type 1 (PCH1).

SMARD1 is a clinically and genetically heterogeneous condition resulting from mutations in the gene coding for the immunoglobulin-binding protein 2 (IGHMBP2), located on chromosome 11ql3. The clinical mimics, in part, that of SMA1 with associated diaphragmatic weakness leading to acute life-threatening respiratory failure. Intrauterine growth restriction, mild hypotonia, weak cry and, in contrast to SMA1, a prominent distal muscles involvement are also present. Peculiarly, respiratory distress in SMARD1 is the consequence of the severe diaphragmatic paralysis with elevation of both hemidiaphragms on chest X-ray, rather than the severe intercostal muscle impairment of SMA1 [35].

PCH1 is a disorder which is not allelic to SMA1 and the pathogenetic mechanism of which is still unknown. Clinical features include arthrogryposis, severe weakness, hypotonia, bulbar signs, severe microcephaly, mental retardation, classically associated with hypoplasia of the cerebellar vermis and, in some cases, cerebellar hemispheres, thin brainstem and pons. The clinical course is progressive [36].

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145

Ocular Malformations

Elena Piozzi and Alessandra Del Longo

145.1 Introduction

There are multiple ocular malformations: some are evident at birth or during the first few months of life, others are revealed at a later stage.

Ocular malformations can be a sign of more complex and general pathology. The importance of early recognition is particularly important for those that can damage vision and/or cause amblyopia (interruption of the development of visual function).

Ocular malformations of the anterior segment are recognised at examination. Malformations of the posterior segment may present either because of their frequent association with anterior malformations or, more often, as a constant or intermittent strabismus.

It is important to be aware that possible serious ocular malformations may be indicated by asymmetry of the eyes, as well as by differences in their color and the sizes of the globes, irises and palpebral fissures. Thus, the role of the pediatrician is crucial in the timely diagnosis of congenital eye pathologies and an appropriate specialist referral [1, 2].

145.2 Eyelid Malformations

Eyelid development occurs during the second month of fetal life. Eyelid opening occurs after about 25 weeks gestation as the development of the eyelid is completed.

Abnormal development may cause anomalies of eyelid position and lid margin.

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145.2.1 Developmental Anomalies

145.2.1.1 Coloboma

Lack of tissue causes a defect of the lid margin, the severity of which is proportional to the size of the defect (Fig. 145.1). It generally affects the nasal half of the superior eyelid and can be isolated or associated with other pathologies (Tessier syndrome, Goldenhar syndrome). Lack of protection by the eyelid and consequent corneal exposure may cause keratitis.

145.2.1.2 Epicanthus

Semi-lunar skin fold with a downwards orientation at the nasal margin (canthus), simulating a convergent strabismus (Fig. 145.2). The recognition of an actual strabismus may be difficult and requires extra care.

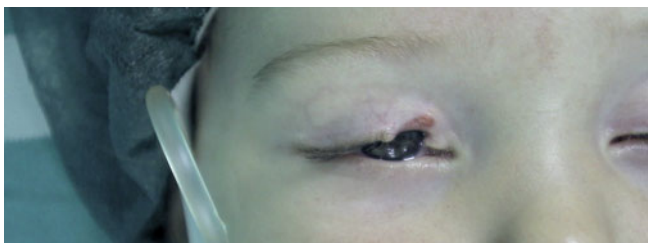

Fig. 145.1 Eyelid coloboma

Fig. 145.2 Epicanthus

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

145.2.2 Anomalies of the Rima (Palpebral Fissure)

145.2.2.1 Distichiasis

This condition is characterized by the growth of a second row of eyelashes. It mostly affects the lower lid. It can be isolated or hereditary (autosomal dominant). If the inner part of the lid margin is affected, distichiasis may cause corneal ulceration, lachrymation and photophobia.

145.2.2.2 Blepharophimosis

This is a complex autosomal dominant syndrome, characterized by a narrow palpebral fissure and inverted epicanthus (Fig. 145.3). It can be associated with strabismus and nystagmus. Treatment is by surgery.

145.2.2.3 Angiomas

Benign vascular tumor that becomes evident a few days after birth and which, depending on their extension and location, may be a cause of total or partial palpebral ptosis with consequent amblyopia if the visual axis is disturbed (Fig. 145.4). Ultrasonography may be useful to establish the degree of extension within the orbit. Generally angiomas are self-limiting processes and resolve spontaneously. Therapy is only indicated when amblyopia is suspected. Therapy is medical (cortisone and beta blockers). Surgery is only indicated for non responsive forms or when vision, including the integrity of the visual axis, is threatened.

145.2.3 Position Anomalies

145.2.3.1 Palpebral Ptosis

Also known as a drooping eyelid (Fig. 145.5). Severe cases can cause a visual disturbance (amblyopia) and abnormal head position. Ptosis may be unilateral or bilateral. Manifests as a difficulty in opening the eyelid from the first days of life. Surgery is reserved for total ptosis. In other cases, therapy is aimed at cure of the amblyopia and surgery, which is done for esthetic reasons, is delayed until children are over 5 years old [3, 4].

145.3 Ocular Bulb Malformations

145.3.1 Anophthalmia

Total absence of the eye (Fig. 145.6).

145.3.2 Microphthalmia

This is a reduced volume of the eye (the normal length of the eye at birth is approximately 17 mm) (Fig. 145.7). The diagnosis is straightforward in monolateral forms because of the inter-ocular difference, more complicated in bi-ocular forms. Microphthalmia is associated with a small eyelid, drooping of the lid and enophthalmos (sunken eye).

If there is anophthalmia and microphthalmia, a prosthesis must be inserted to promote orbital growth [5].

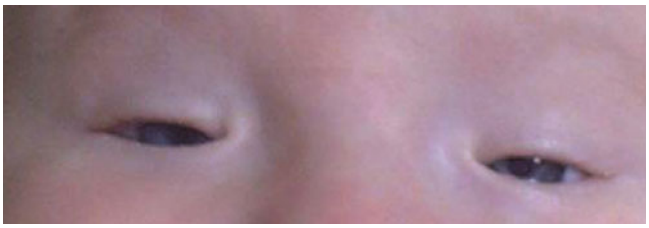

Fig. 145.3 Blepharophimosis

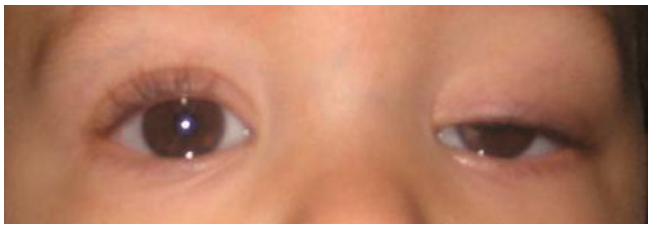

Fig. 145.5 Eyelid ptosis

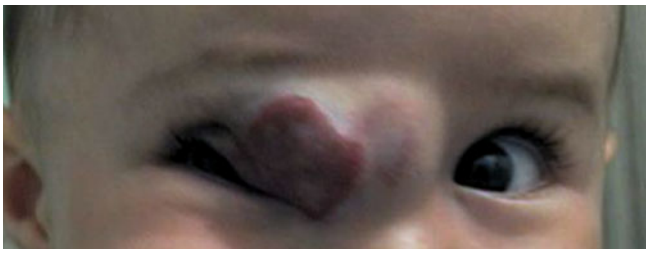

Fig. 145.4 Angiomas

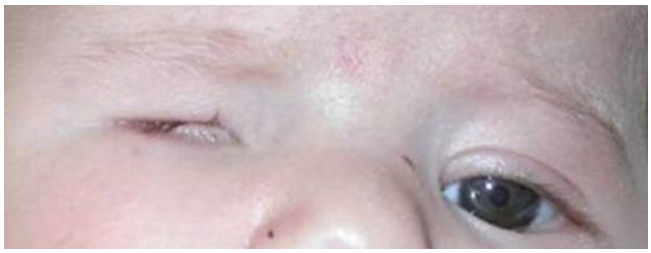

Fig. 145.6 Anophthalmia

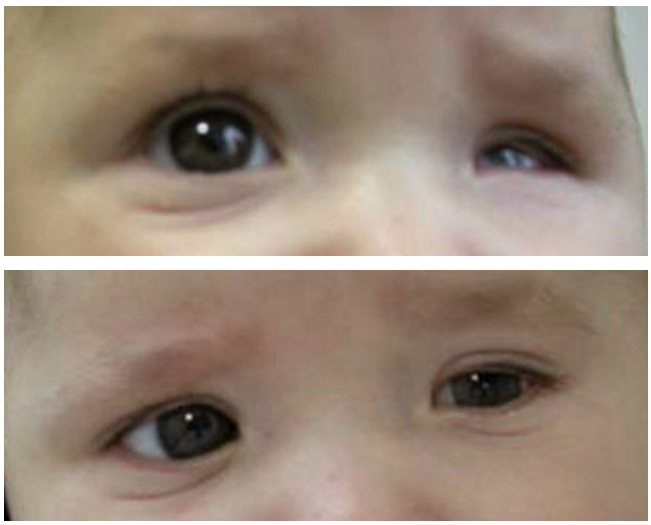

Fig. 145.7 Left eye microphthalmia without and with prothesis

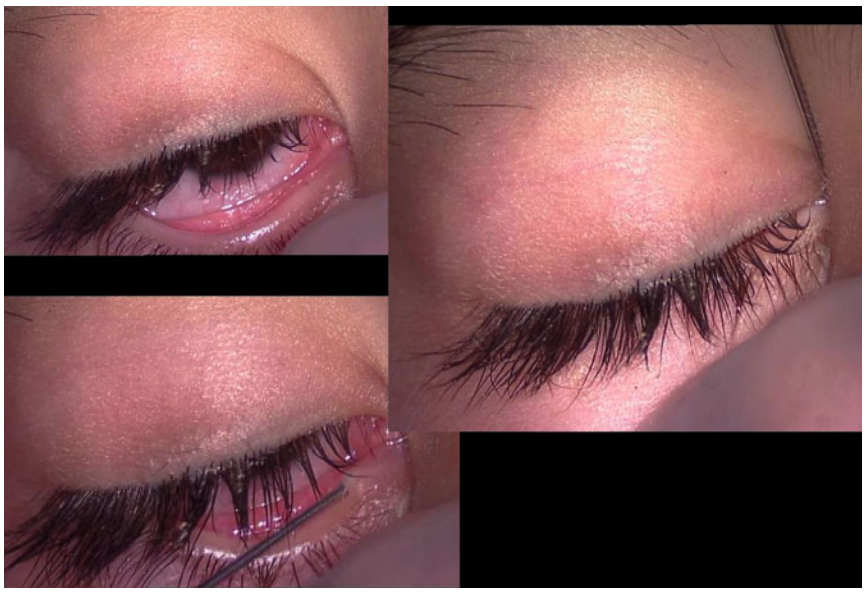

Fig. 145.8 Congenital naso-lacrimal-duct obstruction

145.4 Naso-Lacrimal-Duct Malformation

145.4.1 Congenital Naso-Lacrimal-Duct Obstruction

Epiphora is present from the first days of life. By applying pressure near the lacrimal sac, mucous and purulent material may be discharged from the inferior lacrimal point.

Therapy consists of massage of the lacrimal sac region, cleaning the nose with physiological saline and local antibiotics when needed.

Surgery for cases that do not respond to therapy probing of the lacrimal ducts (Fig. 145.8).

145.5 Corneal Malformations

145.5.1 Size Anomalies

145.5.1.1 Megalocornea

Simple megalocornea is isolated and autosomal dominant. Associated anomalies (megalophthalmos) are X-linked and it is associated with congenital glaucoma (buphthalmos).

Congenital glaucoma is an important differential diagnosis and if enlargement of the eye is suspected, it is important for an ophthalmologist to measure corneal diameter and ocular pressure. Possible watering and photophobia may manifest as a consequence of increased eye pressure.

145.5.1.2 Microcornea

It is a small corneal diameter (normally 9.5-10 mm in the newborn) (Fig. 145.9). May be associated with microphthalmos and other anterior segment abnormalities.

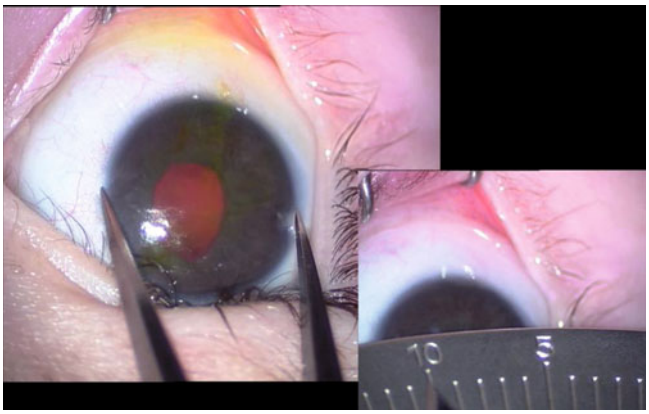

Fig. 145.9 Microcornea

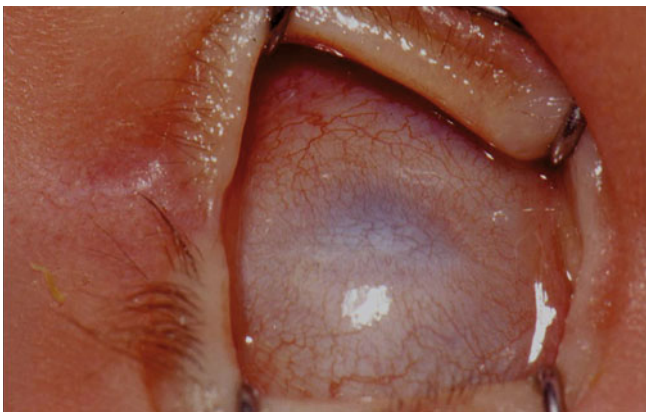

Fig. 145.10 Sclerocornea

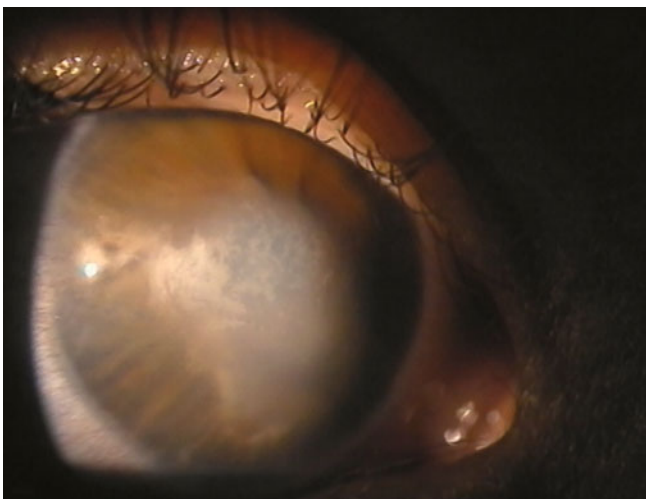

Fig. 145.11 Peter's anomaly

145.5.2 Anomalies of Transparency

These are anomalies of transparency (congenital corneal opacity, sclerocornea (Fig. 145.10), Peter's anomaly (Fig. 145.11), corneal dystrophy and corneal opacifications) and may be associated with metabolic illnesses or congenital infections. Corneal opacifications may be isolated or widespread. Often associated with poor eye opening. May be an indication of serious metabolic illnesses.

145.5.3 Epibulbar Dermoids

Their appearance is similar to a solid white mass in the limbus, or more frequently in the inferior-temporal region (Fig. 145.12). Often associated with the Goldenhar syndrome, tend to involve the cornea causing astigmatism. As they develop, they may involve the optic axis with consequent damage to normal vision. Treatment is by surgery [6].

145.6 Crystalline Lens Anomalies

145.6.1 Epipupillary Membrane Persistence

This is a failure of reabsorption of the epipupillary membrane during normal fetal development.

A veil-like membrane formed by thin vessels and residual mesodermic tissue, partially or totally occluding the pupil, leading to impaired pupillary dilatation and interfering with the visual axis. Treatment is initially medical with the administration of mydriatics. If the membrane is thicker, treatment is by surgery.

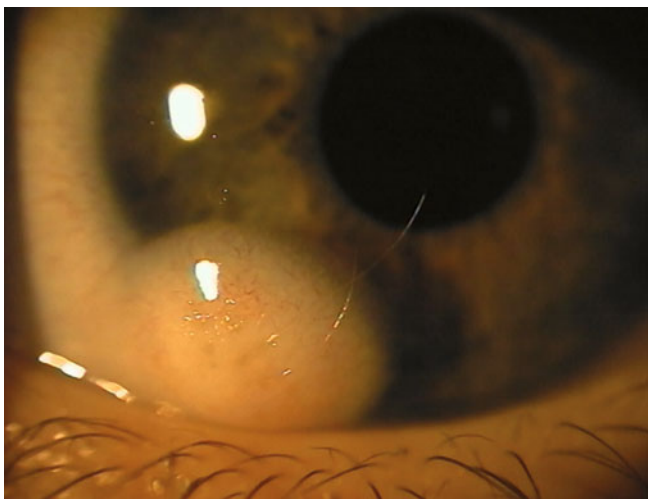

Fig. 145.12 Epibulbar dermoid

145.6.2 Position Anomalies

Dislocation of the crystalline lens from its normal position as a consequence of malformation of zonular fibres of the lens is associated with more generalized syndromes, e.g. Marfan syndrome and Marchesani syndrome. The dislocation can be partial (subdislocation, Fig. 145.13) or complete (dislocation, Fig. 145.14). Since the lens is not in the correct position with respect to the pupil, serious refractive defects and amblyopia may ensue. Subluxation can cause the crystalline lens to fall to the back of the eye (into the vitreous), which results in retinal damage, or to the front (towards the cornea) with endothelial damage. Treatment is surgical.

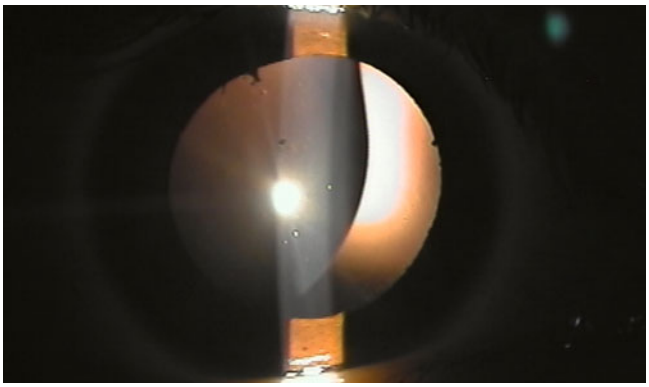

Fig. 145.13 Subdislocation of lens

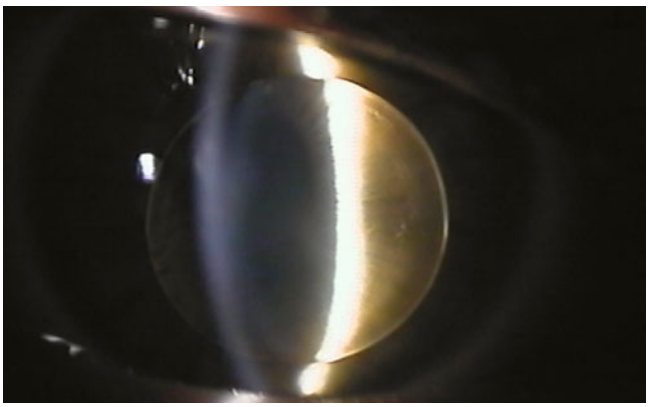

Fig. 145.14 Complete dislocation of lens

145.6.3 Transparency Anomalies

Congenital cataracts can be unilateral or bilateral, isolated or associated with general pathologies, including intrauterine infections (rubella, cytomegalovirus, toxoplasmosis) or metabolic diseases (galactosemia). The lens can be more or less opaque (Fig. 145.15). Total opacification results in the pupil having a characteristic white color (leukokoria) [7].

Treatment depends on the density of the opacification and the degree to which vision is affected. If the cataract is dense, especially if unilateral, surgery should be performed as soon as possible. The operation is aimed at removal of the opaque lens with the possible implantation of an intraocular lens. Treatment aimed at preventing amblyopia is important after surgery.

145.6.4 Malformative Glaucoma

This is a developmental abnormality of drainage, causing increased intraocular pressure and an enlarged eye (buphthalmos) (Fig. 145.16). Alterations of the anterior segment (corneal edema, striated lines) may be present at birth or manifest later. Physical signs include severe photophobia and watering of the eye.

Early diagnosis is essential for a good outcome.

Glaucoma is an hereditary disease. Nowadays a study to discover the gene mutation (CYP1B1, MIOC) is being undertaken in children affected by congenital glaucoma.

Treatment can be medical or surgical.

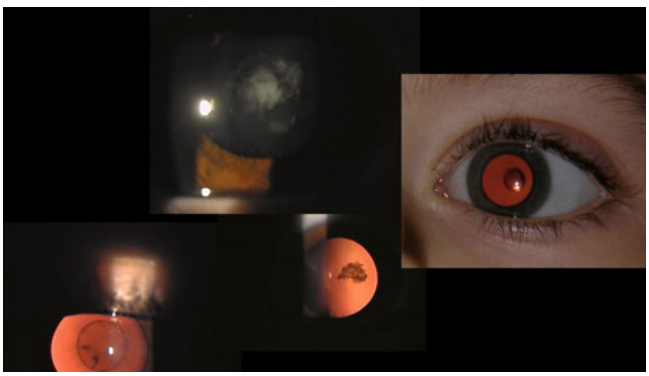

Fig. 145.15 Congenital cataracts

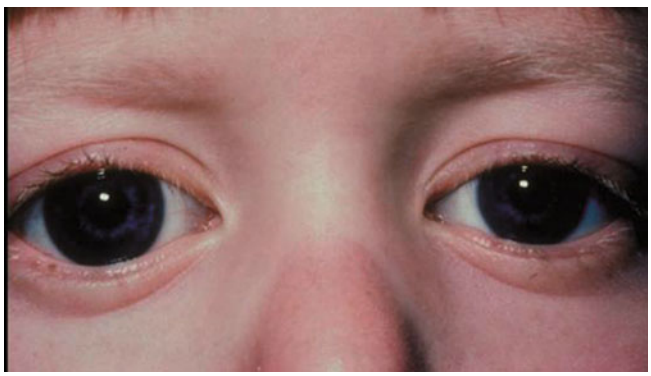

Fig. 145.16 Buphthalmos

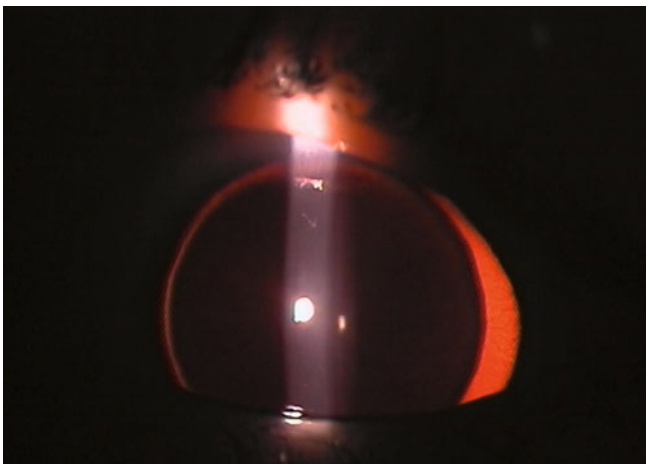

Fig. 145.17 Aniridia

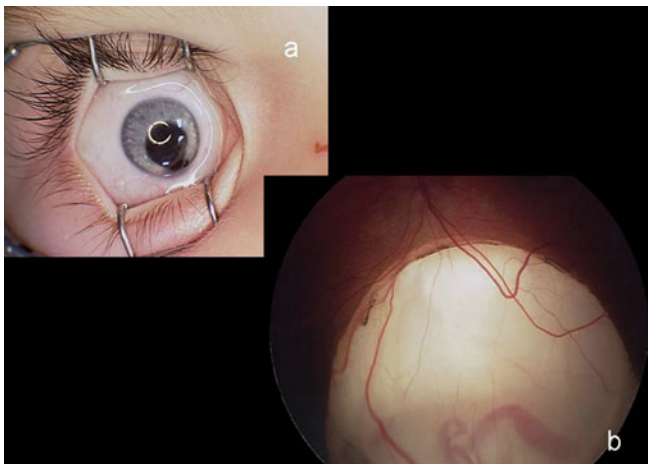

Fig. 145.18 Iris and retinochoroidal coloboma

145.7 Congenital Anomalies of the Iris and Pupil

145.7.1 Aniridia

This is a total or partial absence of the iris, normally bilateral (Fig. 145.17). It is associated with nystagmus and photophobia. It may be inherited with dominant autosomal transmission. Aniridia can be associated with glaucoma.

An eye test for aniridia always includes measurement of intraocular pressure. In isolated forms it is frequently associated with nephroblastoma or Wilms' tumor (abdominal ultrasonography is mandatory).

Vision correction and the use of tinted lenses can improve vision for those patients [8].

145.7.2 Iris Coloboma

Coloboma of the iris is a deficiency of part of the iris (normally in the inferior sector) (Fig. 145.18). It is due to abnormal closure of the fetal fissure. Close to the coloboma, the pupil is irregular with an elongated oval appearance. The malformation is normally reported by the mother who notices that "the pupil looks strange" whilst she is breast-feeding. This anomaly may be associated with colobomas of the posterior segment of the eye (choroid-retina).

145.7.3 Leukokoria

A white pupillary reflex signifies a cataract or malformation and congenital vitreoretinal disease.

145.7.4 Malformations of the Posterior Segment

Malformations of the posterior segment such as a coloboma (Fig. 145.18), congenital malformations of the optic nerve and of the vitreo-retina can manifest mainly because of constant or intermittent strabismus. If parents report repeated strabismus, the pediatrician should refer the patient for ophthalmological review.

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Retinopathy of Prematurity

José Carlos Rivera, Elsa Duchemin-Kermorvant, Allison Dorfman, Luis M. Ospina and Sylvain Chemtob

146.1 Introduction

Retinopathy of prematurity (ROP) is an ocular disease that occurs in premature infants and affects the blood vessels of the developing retina. It is characterized by the onset of visible vascular abnormalities in the second or third month after birth. The pathogenesis of ROP is related to many causative factors, like low birth weight, low gestational age, supplemental oxygen therapy, and some lines of evidence suggest the role of a genetic component. ROP is mild and undergoes spontaneous regression with no visual sequels in the majority of affected infants. However, progression to advanced ROP does occur in a significant number of infants and can lead to severe visual impairment and even complete unilateral or bilateral blindness in some cases. In general, more than 50% of premature infants weighing less than 1250 g at birth show evidence of ROP, and in approximately 3% of children it undergoes abnormal retinal vascular development, neovascularization, and, in its more severe forms, traction retinal detachment [1]. These injuries may occur despite the aggressive interventions currently available, such as cryotherapy or laser photocoagulation for this sight-threatening disease.

ROP has been recognized since 1942 as a blinding disease of the very premature infant [2] and the incidence of blinding forms of retinopathy has varied greatly over the intervening decades. Over more than half a century of intense clinical and laboratory research great efforts have been made in developing effective therapies for severe stages of ROP, and the understanding of the nature of this disease has improved tremendously. But modest progress has been made in truly understanding the cellular and molecular mechanisms causing this pathology and even less in developing effective prophylactic treatments. At present, the problem increases worldwide with the technological advances in neonatology leading not only to an increased survival rate of very low birth weight infants, but also to a correspondingly increased incidence of ROP. This, in turn, has provided a major challenge to all physicians treating the premature infant and has created renewed interest in the pathogenesis, prevention, and treatment of ROP. However, the central mysteries of this disease have not been adequately unraveled to effectively eradicate unfavorable visual outcomes and the efforts continue. The purpose of this chapter is to present current knowledge about ROP, demographic incidence, risk factors and its more recent treatment strategies, as well the possible promising future therapies against this serious disease.

146.2 Historical Background

Prior to the 1940s, ROP was an unknown disease because severe prematurity was often fatal. The first case of ROP was described in Boston by Dr TL Terry in 1942 [2]. His initial report referred to this new disease as possible fibroblastic overgrowth of the persistent tunica vasculosa lentis. In a second report [3], containing several pathological specimens in the cicatricial end stages of the disease with multiples complications, Terry clearly misinterpreted the involved pathophysiology. He did not recognize the retina as the source of the problem and still linked it to the rare congenital birth defect of persistence of hyaloid artery and tunica vasculosa lentis. However, he did recognize the salient epidemiology and showed that the disease occurred much more frequently in infants born extremely prematurely [3]. In this same report Dr Harry K Messenger, a colleague of Terry, suggested the term "retrolental fibroplasia" that was used for about 40 years and that described the end-stage of the disease in which a white, vascularized plaque could be seen behind the lens in an eye that was often completely blind. At that time, there was inadequate technology to carefully monitor oxygen levels

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G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

in neonatal intensive care units and high doses of oxygen were being administered to premature infants without monitoring; the pulse oximeter was not yet available. In fact, during the next 12 years following the first report, more than 10,000 premature infants in North America were blinded before it became appreciated that the outbreak was related to the use of supplemental oxygen [4]. Much of our understanding of ROP can be credited to Kate Campbell from Melbourne, Australia who compared the incidence of ROP at hospitals that charged for oxygen use and hospitals that did not [5]. This investigator found that the incidence of ROP was higher at those hospitals where the oxygen was free and use was unrestricted. Furthermore, with the advent of pulse oximetry in the 1970s and beyond, oxygen administration became more controlled, and it was thought that ROP would disappear. Unfortunately, this proved to be untrue. As neonatologists got better at saving tinier and sicker babies, the rate of ROP rose again to frighteningly high levels. Soon, small gestational age and very low birth weight correlated more strongly with occurrence of ROP than did oxygen administration. During the 1980s and early 1990s a major understanding in the physiopathology disease progressed with the use of animal models [6, 7], suggesting that the degree of prematurity of the eye at birth was the critical and necessary risk factor for the disorder and that ROP goes through several well-defined stages before getting to the irreversible retrolental fibroplasia. During the early 1980s the term of retrolental fibroplasia was renamed to retinopathy of prematurity, and with new knowledge of ROP pediatrics, ophthalmologists and neonatologists united to find a treatment. The first step was the development of a common nomenclature, followed by a natural history study and a treatment protocol. In 1984, the International Classification of Retinopathy of Prematurity (ICROP) was developed [8]. Despite the efforts, no treatment for ROP was available for several years. Major advances in ROP treatment came in the 1980s and 1990s, when cryotherapy and laser photocoagulation, which consists of destroying the portion of the retina that is unvascularized in order to preserve the rest of it, showed to be partially effective in preventing blindness in ROP infants. However, these surgical procedures are sometimes uncomfortable or painful and can only reduce the incidence of blindness by 25% in infants with late-stage disease, moreover these patients often still have poor visual acuity after treatment [9]. Nowadays, the fight against ROP continues generating new information including possible genetic predisposition [10] and the role of angiogenic growth factors, such as insulin-like growth factor (IGF-1) [11], or vascular endothelial growth factor (VEGF) [12], that are involved in the development of pathological retinal neovascularization. Over this decade new agents targeting VEGF have become commercially available for intraocular use. Development of pharmacological strategies for treating ocular neovascularization has revolutionized the care of some neovascular ocular diseases and has great potential for ROP. The use of preventive and less destructive therapies for this pathology such as nutritional supplements, including forms of vitamin E [13], amino acids [14], or omega 3 fatty acids [15], would be much more desirable, however, understanding the molecular basis of the disease is important to the development of new medical interventions or therapeutic treatments.

146.3 Epidemiology

It is estimated that of the 45 million people who are blind worldwide in the 2000s, 1.4 million are children from middle income and low-income countries, the majority of whom live in the poorest regions of Africa and Asia [16]. In 1995, ROP accounted for 10.6% of cases of blindness in children in schools for the blind in South Africa [17]. However, ROP is not considered a significant cause of childhood blindness in very poor countries due to the low survival rate of premature infants [18]. Yet, ROP is emerging as a major cause of childhood blindness in middle-income countries. For example, a study from 1997 through 2005 showed that of the 34 million live births analyzed in the United States, 58,722 newborns were identified with ROP. This study showed that the incidence of ROP has increased from 0.12% to 0.17%, and that in the patient population at risk for ROP, it has doubled from 7.35% to 15% [19]. Furthermore, almost half of the world's 50,000 children who are blind from ROP live in Latin America [20]. The World Health Organization estimates that 24,000 of the 100,000 visually impaired infants in Latin America are attributable to ROP [21, 22]. An observational study [23] comparing characteristics of infants who develop severe ROP from countries with low, moderate and high levels of development showed that larger, more mature infants are at risk in the less developed countries. In the developing countries, the mean birth weight of infants with severe ROP ranged from 903–1527 g, compared with 737–763 g in the highly developed countries. The mean gestational age of infants with severe ROP from the developing countries was 26.3–33.5 weeks, compared with 25.3–25.6 weeks in highly developed countries. In nearly all of the middle-income countries, infants who developed severe ROP had a gestational age of more than 32 weeks and weighed more than 1500 g. This fact demonstrates the inadequacy of the current screening guidelines used in developed countries when evaluating infants from middle-income countries [24, 25].

While exposure to an oxygen-rich extrauterine environment is clearly identified as a major factor in the genesis of ROP (see below), it is not the only predisposing factor. Interestingly, there are many premature infants that are treated with supplemental oxygen who will never end up developing ROP, while other children that are nearly full-term who receive little or no treatment will eventually present with the disease [26]. Not only are low birth weight and low gestational age correlated with the incidence of ROP but ethnicity is also a factor that has been reported to significantly influence its epidemiology [27]. Findings by some have suggested that fundus pigmentation might play a protective role against the progression

to severe ROP given the greater incidence of threshold ROP in Caucasian infants compared to African-American infants [27, 28]. However, others have reported an even greater incidence in the onset of threshold ROP exists in Alaskan natives, a darkly pigmented race, when compared to other racial subgroups [29, 30]. There are also many indications that the patient's genetic make-up plays a role in whether ROP develops, and how severe it will be. Although ROP occurs in all populations, it long has been noted to occur in its most severe form less frequently in blacks than in whites. In the CRYO-ROP study, 3.2% of black infants developed threshold ROP compared with 7.4% of white infants. In black South African infants, a similar rate of 3.2% in infants born at less than 1251 g was found [31]. This is unlikely to be caused by environment, because it is constant across continents. It could be a protective effect of increased retinal pigment, but it also could be because of differential genetic effects, more common in one population than another. Males also were found to have a higher risk than females in the CRYO-ROP study. And in several studies, variations in the Norrie disease gene, a gene responsible for an X-linked form of congenital retinal detachment or dysgenesis were more common in infants with severe ROP than in those in whom the disease resolved on its own [10, 32]. Each person carries genetic predispositions to certain disorders, but less widely understood are genetic proclivities toward outside effects, trauma, hypoxia, premature birth among them. Further investigation into the potential factors that might contribute to these discrepancies, including genetics, socio-economic status and dietary intake, is warranted.

146.4 General Definition

Retinopathy of prematurity (ROP) is a blinding eye disease of premature infants that affects more than 80% of babies born with birth weight less than 1000 g. It is a disease confined to the immature retinal vascular system and it is characterized by disorganized growth of retinal blood vessels, which may result in scarring and retinal detachment. ROP can be mild and undergoes spontaneous regression with no visual sequelae in the majority of affected infants. However, progression to advanced ROP does occur in a significant number of infants and can lead to severe visual impairment and even complete unilateral or bilateral blindness in some cases. Many causative factors can contribute to the development of ROP including low birth weight, low gestational age, supplemental oxygen therapy and some lines of evidence suggest the role of a genetic component.

146.4.1 Progression of ROP

Retinal blood vessel development commences around 16 weeks of gestation. The nasal retina is vascularized by 36 weeks gestation and the temporal retina by 40–45 weeks [33]. Therefore, the retinas of premature infants are incompletely vascularized. Two phases of ROP are characterized [34]. The first phase begins when retinal vascular growth ceases after premature birth. The relative hyperoxic environment causes downregulation of the major hypoxia-triggered vascular endothelial growth factor (VEGF) resulting in vaso-obliteration of the developing retinal capillaries. The ensued relatively vascular-depleted retina becomes increasingly hypoxic and a second phase begins. In this phase VEGF and other growth factors are upregulated, leading to a proliferation of abnormal neovascularisation with the development of stage 3 ROP. VEGF is an important hypoxia regulated factor in the development of ROP. Studies have shown that serum levels of VEGF are higher in infants with stage 3 and threshold ROP compared to those with less severe disease [35].

The time of the appearance of ROP is determined both by the immaturity of the child at birth and by perinatal events that are associated with increased incidence of ROP. In full-term infants, ROP almost never develops when oxygen is delivered in high concentration. Also, when oxygen is monitored carefully and controlled in mildly preterm infants with zone II or III disease, the retinal vasculopathy is usually mild; this inference has been corroborated by a number of human studies wherein lower O_2 saturation revealed decreased rates of ROP, without causing increased neurologic complications (see § 146.5). Conversely, the risk of ROP rises in very premature infants whose eyes have large areas of avascular retina.

146.5 Pathogenesis

The vasculature of the human retina begins to develop by approximately 16 weeks gestation and reaches the periphery at near-term gestation; accordingly, in the premature infant retinal vascularization is incomplete. The endothelial cells committed from angioblasts lead the way in response to increasing metabolic demands of the developing retina [36]. Pericytes develop later and their absence facilitates angiogenic stimuli to induce endothelial cell proliferation [37]. This process of angiogenesis occurs as expected in part through expression and actions of VEGF [38].

Increased retinal oxygenation is an important factor in the pathogenesis of ROP [39–41]; in utero the immature subject is exposed to much lower levels of O_2 than postnatally. Recent studies [42–48] have concluded that the rate of ROP can be reduced by limiting O_2 administration (Table 146.1); this of course needs to be done cautiously. In contrast to the adult [49, 50] the immature newborn is unable to regulate O_2 delivery (principally from the highly perfused choroid) to the inner retina [51–54]. In response to the ensued retinal hyperoxia, not only the particularly vulnerable endothelial cells at the vascular front degenerate, but also a marked involution

Table 146.1 Rate of ROP in infants exposed to different hemoglobin O_2 saturations (Data collection: retrospective)

Reference	BW	O_2 sat (>90%)	O_2 sat (82–90%)
Tin et al, 2001 [42]	<1500 g (n = 210)	13.5% (threshold)	6.3% (threshold)*
Chow et al, 2003 [43]	<1500 g (n = 447)	4.5% (threshold)	1.3% (threshold)*
Wright et al, 2006 [44]	<1500 g (n = 350)	7.3% (threshold)	1.3% (threshold)*
VanderVeen et al, 2006 [45]	<1250 g (n = 323)	16.7% (threshold)	4.2% (threshold)*
Deulofeut et al, 2006 [46]	<1250 g (n = 502)	36% (ROP = 2)	25% (ROP = 2)*
Wallace et al, 2007 [47]	<1250 g (n = 89)	18% (ROP < 2) 25% (ROP > 3)	14% (ROP < 2) 22% (ROP > 3)

* $p < 0.05$.

of the choriocapillaris in the central choroid was recently reported as an important contributor to central photoreceptor compromise in the OIR/ROP [35, 55, 56]. This endothelial cytotoxicity embodies the vaso-obliterative phase of ROP [57], and impacts on neovascularization (as well as on retinal function secondary to the ensued ischemia [58, 59]).

A number of factors participate in the vaso-obliteration that precedes the pre-retinal neovascular phase: a) O_2-dependent suppression of the important endothelial growth/survival factor VEGF through stabilization of hypoxia-inducible factor (HIF) [60, 61]. b) Decreased expression of the nutrition-dependent factor insulin-like growth factor (IGF-1). c) Inability of the newborn to autoregulate oxygen delivery compared to the adult. d) Increased generation of reactive species of oxygen and their products, notably thromboxane A_2, platelet-activating factor (PAF), lysophosphatidic acid (LPA) and trans-arachidonic acids [62]. e) Increased vulnerability of the neuroretinal endothelium to oxidant stress (more so than neurons and glia).

The increased generation of reactive oxygen species is the result of an incompletely developed anti-oxidant potential in the immature subject and a tissue (the retina) highly prone to peroxidation due to a high content of polyunsaturated fatty acids; in fact the retina contains the highest concentration of long-chain fatty acids in the organism. Antioxidants are molecules that can safely interact with ROS and terminate the chain reaction before vital molecules are damaged. The concentration of all the major components of the antioxidant systems in neonates, which include heme oxygenase-1, metallothionein, Cu-Zn superoxide dismutase, catalase, vitamins C and E, and glutathione peroxidase, are reduced in retinal tissues. Hence, a limited ability of the newborn to autoregulate oxygen delivery, the abundant unsaturated fatty acids in retina, and the reduced antioxidant systems, renders the ocular vasculature of the newborn more vulnerable to oxidative damage.

146.5.1 Oxygen-Independent Factors and ROP

146.5.1.1 Hypercapnia

In addition to hyperoxia, other factors regularly encountered in neonates with bronchopulmonary dysplasia, such as high carbon dioxide (CO_2) tension (hypercapnia) and associated acidosis, have also been shown to play a role in the development of ROP [63]. Interestingly, ischemia also results in a concomitant elevation in CO_2 [64]. This local tissue hypercapnia arises as a consequence of 1) decreased waste removal, 2) continued aerobic CO_2 production until O_2 stores are depleted, and 3) increased anaerobic CO_2 generation from the bicarbonate buffering of hydrogen ions (derived from lactic acid breakdown and high-energy phosphate hydrolysis). The specific consequences of hypercapnia have generally been overlooked with respect to neovascularization.

Exposure of endothelial cells to hypercapnia has been shown to inhibit their proliferation, migration and differentiation into capillary-like structures and to decrease their viability, apparently through cytotoxic nitration [65]. Hypercapnia also attenuates developmental retinal neovascularization with acute and prolonged exposure. Likewise, in a mouse model of O_2-induced retinopathy, retinas are unable to revascularize as efficiently when CO_2 levels are concomitantly elevated [65].

146.5.1.2 Insulin-like Growth Factor-1 (IGF-1)

Of all contributors, prematurity remains the greatest risk for ROP, suggesting that certain key factors present in utero that partake in normal fetal development are lacking in preterm infants. This hypothesis was explored in the seminal works of Lois Smith on the role of deficiency of the major growth factor, IGF-1 in premature subjects [9]. Plasma levels of IGF-1 rise with gestational age and considerably augment during the third trimester of pregnancy (when premature infants predisposed to ROP are born). Importantly, IGF-1 decreases postnatally, thus underscoring the placental and amniotic source of the factor. In human subjects, low IGF-1 serum levels directly correlate with the severity of ROP, and interestingly may also account for abnormal brain development where retinal vessel growth was retarded, akin to patterns noted in premature babies with ROP [66]; interestingly, IGF-1 binding protein, IGFB3, was also found to be decreased in premature infants, and may also contribute to retinal vessel depletion. It was also found that IGF-1 modulates VEGF actions. On one hand, IGF-1 curtails protective actions of VEGF during the vaso-obliterative phase. Subsequently, when retinal ischemia develops and VEGF levels rise, IGF-1 plays an essential role as a permissive factor allowing VEGF to exert its vasoproliferative actions. Hence, early on IGF-1 depletion participates in vaso-obliteration and later IGF-1 repletion contributes to the aberrant pre-retinal neovascularization. Accordingly, clinical

trials are being undertaken to address the merits of treatment with IGF-1 at an early stage to prevent the vaso-obliteration in the premature infants [9]. Based on these and animal observations the question of poor weight gain in premature infants at risk of ROP has been raised. In this context, it was recently found that inadequate weight gain during the first few weeks of postnatal life is associated with more severe ROP. Accordingly, an algorithm based on weight gain and IGF-1 was developed as a predictor of ROP (WINROP). Using serial weight and IGF-1 measurements in a cohort of 50 premature babies, WINROP predicted all infants who later developed ROP by a mean age of 10 weeks [67]. Moreover, measurements of weight gain (and exclusion of IGF-1) was sufficient to demonstrate in a cohort of 351 patients that insufficient weight gain could predict all infants who required treatment and 75% of babies who did not develop ROP [68].

146.6 Classification

Prior to the early of 1980s, most ophthalmologists recorded observations about the natural course of ROP using different classification systems. Though these systems were similar in the extreme degrees of ROP, moderately severe grades of retinopathy had different emphases that made discussion of ocular findings and comparison of treatment effects difficult. This situation was radically altered with the introduction of the International Classification of Retinopathy of Prematurity (ICROP) in 1984 [8]. This classification was a group effort of 23 ophthalmologists and ophthalmic pathologists from 11 countries. The ICROP group suggested four major components for an international classification, including: 1) localization (Zones I, II and III) of the retinopathy between the ora serrata and the optic disc, 2) severity (Stages 0-5) of disease at the junction between the vascularized and non-vascularized retina, 3) extent of involvement of disease (given in clock hours 0 through 12) at the junction, and 4) presence or absence of plus disease, defined as abnormal dilatation and tortuosity of the vessels of the posterior pole of the eye. The major theme of the classification was that the more posterior, the more severe and more extensive retinopathy was the most likely to have the worst long-term outcome for the child. All these components have greatly enhanced the study of the course and the treatment of both the acute and cicatricial phases of the disease.

146.6.1 Zones in ROP

The area of the retina affected by ROP is divided into three zones (Fig. 146.1). Zone I, the most posterior, is the area bound by a circle that has the disc at its center and a radius of twice the disc to foveal region distance. ROP develops in this zone if the retina in this area is most underdeveloped. Zone II is concentric around Zone I and having a radius defined from the disc to the nasal ora serrata. Zone III is the crescent of peripheral retina from the edge of Zone II to the ora serrata. Thus, ROP that extends for 12 clocks hours (360°) must occur in Zone I or II. Disease in Zone I is more severe compared with disease limited to Zones II or III.

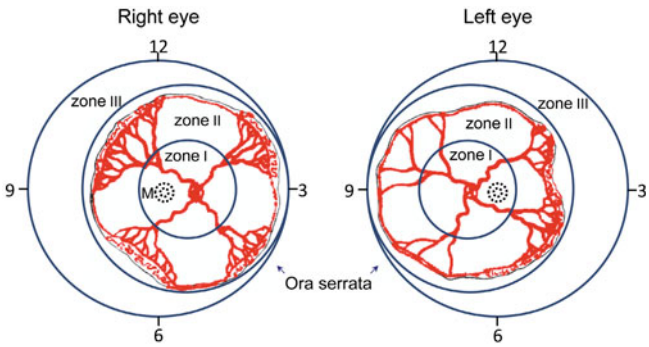

Fig. 146.1 International Classification of Retinopathy of Prematurity Zones and Stages. This figure shows threshold disease in both eyes, with dilation and tortuosity of the posterior pole vessels. Modified from [73]

146.6.2 Stages in ROP

ROP is divided into stages 0 to 5.

- *Stage 0* is the first stage of this disease characterized by an incomplete vascularization of the retina, leaving avascular tissue in a doughnut shape, or a temporal ellipse, around the optic nerve.
- *Stage 1* is uniformly recognizable as a white line develops at the junction between the vascularized and non-neovascularized retina.

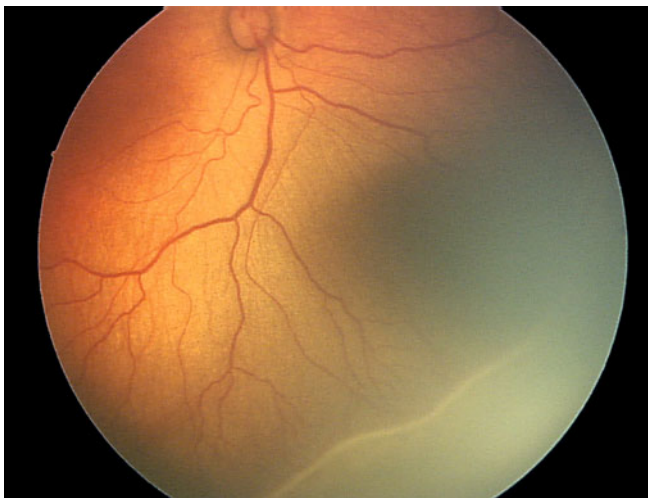

Fig. 146.2 ROP stage 2, the vascular and avascular retina are separated by thick ridge

- *Stage 2* occurs when the line develops height and width. Stages 1 and 2 do not usually require treatment in the majority of the cases (Fig. 146.2).
- *Stage 3* is characterized by extension of fibrovascular proliferation into the vitreous cavity (Fig. 146.3). The presence of neovascular tufts, called "popcorn" were described as being present in active disease [69]. Some cases can present an ominous sign called plus, in which the retinal vessels around the optic nerve and in severe cases on the iris also, become engorged and tortuous. The natural history study showed that if an eye developed five contiguous or eight cumulative clock hours of stage 3 in zones I or II in the presence of plus, there is a 50% chance of retinal detachment with a poor visual outcome [70]. This point in the disease was termed threshold. Approximately 95% of the babies who require laser or cryotherapy develop threshold disease between 32 and 42 weeks after conception. Lower zone and higher stage correlates in general with younger and smaller premature birth and higher risk (e.g., zone I stage 3 is more ominous than zone III stage 1).
- *Stage 4* ROP is the beginning of the blinding consequences of ROP. The neovascular ridge contracts, pulling the retina up with it and causing partial retinal detachment. This stage is divided in Stage 4A if there is a partial retinal detachment not involving the macular region or Stage 4B (Fig. 146.4) if the partial retinal detachment involves the macula. At this stage, the retina may sometimes reattach spontaneously, however, in many cases the retinal detachment, which is unlikely to regress, requires treatment. Treatment modalities are cryopexy for shallow retinal detachment, or scleral buckling for more advanced cases. Stage 4 may progress quickly in some babies and its treatment has frequently been unsuccessful leading to stage 5 ROP.
- *Stage 5* ROP shows a total retinal detachment and is the end stage of the disease. Stage 5 in its most severe form shows a dense white membrane or scar behind the lens of the eye. The detached retina is adherent to this scar tissue. The condition was previously called retrolental fibroplasia (RLF), because the scar is white and visible through the pupil [3]. Spontaneous reattachment of the retina rarely happens in stage 5 ROP. If the eye is left alone at this stage, the baby becomes permanently and often totally blind in both eyes. In addition, some babies develop high intraocular pressure, causing glaucoma. Others develop problems leading to clouding of the cornea.

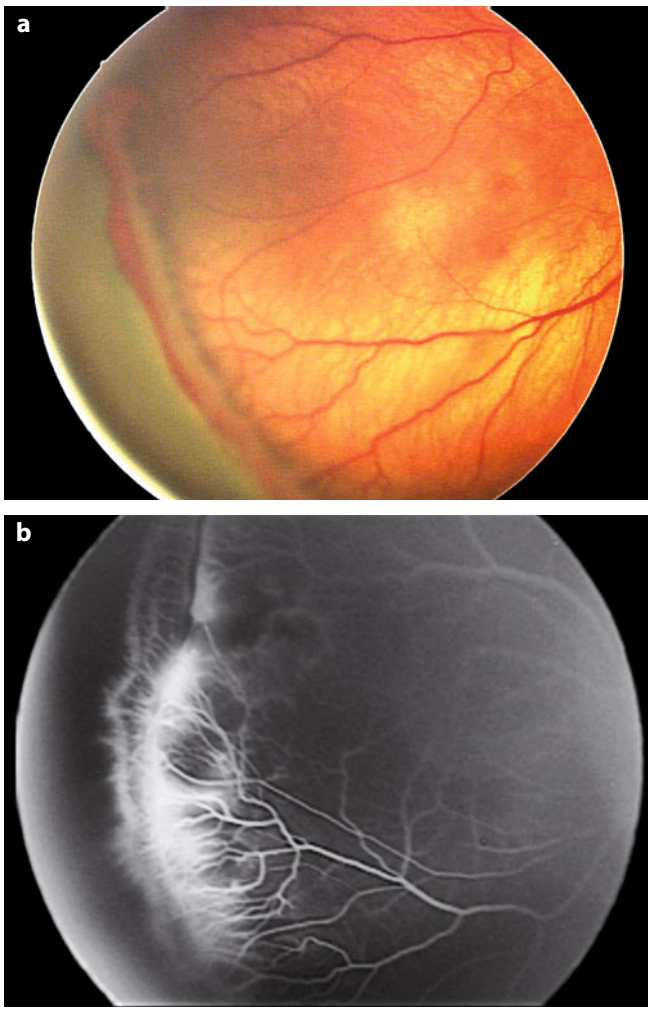

Fig. 146.3 Funduscopy (**a**) and fluoroangiography (**b**) show how neovessels grow towards the vitreous posterior to the white line. (Fluoroangiography courtesy APEC, Association to Prevent Blindness in Mexico)

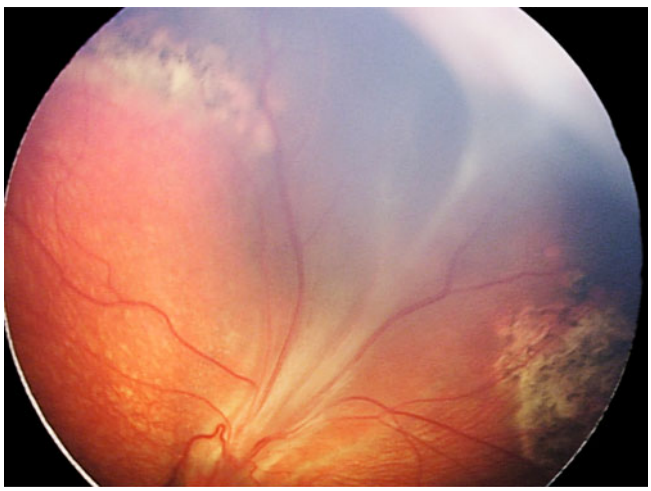

Fig. 146.4 ROP in stage 4B, the fovea becomes involved in a partial retinal detachment. (Courtesy APEC, Association to Prevent Blindness in Mexico)

146.6.3 Severe ROP

146.6.3.1 Plus Disease

Abnormal dilatation of retinal veins with florid abnormal new vessels is called Plus disease, a sign of very poor prognosis in ROP. It is characterized by progressive vascular incompetence

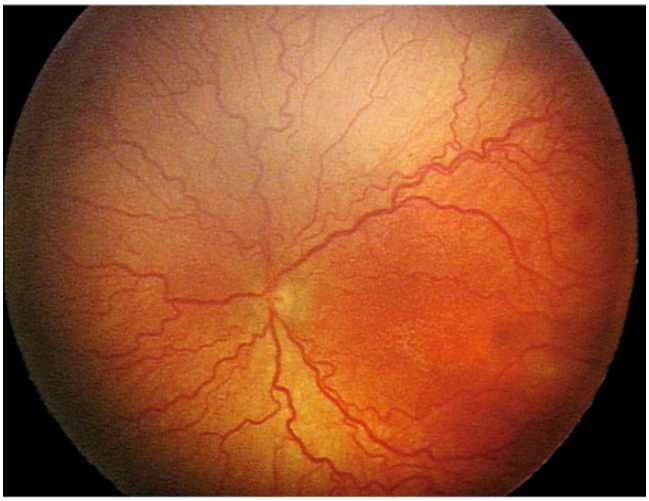

Fig. 146.5 Plus disease, increasing dilatation and tortuosity of veins and arteries of the posterior pole

with increasing dilatation and tortuosity of veins and arteries of the posterior pole, as shown in Fig. 146.5. Also, frequently seen in moderate to severe plus disease are iris vascular engorgement, pupillary rigidity, vitreous haze, and increasing dilatation and tortuosity of the peripheral retinal vessels [71].

146.6.3.2 Rush Disease

Timing is one of the important factors that makes treatment successful in ROP, because the disease can advance very quickly and delayed treatment often reduces the chances of success. The rapidly progressing ROP is called Rush disease, and it is usually associated with very extensive or aggressive growth of abnormal blood vessels [72].

146.6.4 New Classification

The Revised International Classification [73] introduced the new form which concerns premature infants with extremely low GA, defined as aggressive posterior ROP (AP-ROP) and a description of an intermediate level of plus disease: pre-plus

The criterias that characterize AP-ROP are the same: posterior location (zone I), increasing dilatation and tortuosity of the posterior arteries and veins in all four quadrants and shunting from vessel to vessel, within the retina and not solely at the junction between the vascularized and non vascularized retina and a flat network of neo-vascularization. Timing is one of the important factors that make the treatment successful in ROP, because the disease can advance very quickly and delayed treatment often reduces the chances of success. The rapidly progressing ROP is usually associated with very extensive or aggressive growth of abnormal blood vessels [74].

The pre-plus disease indicates vascular abnormalities of the posterior pole that are insufficient for the diagnosis of plus disease, but that demonstrate more arterial tortuosity and more venous dilatation than normal. This situation requires attention to progress a frank plus.

146.6.5 Diagnostic Examination

Indirect ophthalmoscopy with 25–28 D lens is the correct modality to observe the premature retina. This examination is fundamental, it needs papillary dilatation with eye drops which combines 2.5% phenileprine and 1% tropicamid, 3 times in each eye one hour before examination.

In the last years, RETCAM is a new device, which permits the taking of pictures of the retina and allows comparisons between experts.

The fluorescein angiography is important to complete the ROP valuation especially in forms that need treatment. After treatment RETCAM examination and fluorescein angiography help to determine if treatment is complete or not.

146.7 Clinical Presentation

Much of our knowledge regarding the clinical presentation of ROP has been obtained from follow-up data from infants enrolled in the no-treatment arm of prospective randomized trials (natural-history cohorts), mainly the CRYO-ROP and ETROP studies. The CRYO-ROP Study was a multi-center randomized blinded trial designed to evaluate the risks and benefits of trans-scleral cryotherapy [74]. It enrolled 4099 infants weighing less than 1250 g at birth who were born between January 1986 and November 1987. The ETROP Study was a multi-center, randomized trial conducted 15 years later and designed to investigate the potential benefits of early (prethreshold) treatment versus conventional (threshold) treatment in high-risk prethreshold eyes [75] during which 6998 infants born between October 2000 and September 2002 with a birth weight of less than 1251 g were screened for ROP.

146.7.1 Onset

146.7.1.1 Age at Onset

While the risk of developing ROP is influenced by several factors including gestational age and neonatal events, the time at which ROP develops appears to be rather constant and closely related to postmenstrual age [76, 77]. Data from the CRYO-ROP study showed that retinal conditions indicating a risk of poor outcome are not observed before 31 weeks'

postmenstrual age (PMA) or 4 weeks' chronologic age in 99% of infants [78]. In the ETROP and CRYO-ROP studies, the median PMA at first identification of any ROP was 34.1 weeks and 34.3 weeks, respectively [79]. However, a study by Subhani et al suggests that ROP onset is slightly accelerated in the most immature infants thus occurring at a slightly earlier postmenstrual age [77]. ROP can also develop after 37 weeks; it is then most unlikely to develop into disease requiring treatment [78].

146.7.1.2 Site of Onset

ROP develops initially within the peripheral retina, more frequently in the nasal retina in the most immature infants [76]. However, over time location of disease can change [80], maybe due to ocular growth. The risk of developing significant ROP depends on the location of incomplete vascularization by zone. Zone I ROP is high-risk disease, while zone III has very low risk and zone II is intermediate.

146.7.2 Natural Evolution

Despite advances in neonatal care, the CRYO-ROP and the ETROP natural-history studies have shown little change in the natural evolution of ROP [79].

146.7.2.1 Regression

In the CRYO-ROP study, ROP was found to regress with vascularization of the retina growing into zone III in 80% of cases [74]. The mean age at which ROP began to involute was 38.6 weeks PMA [80]. Involution was observed in 90% of infants before 44 weeks PMA, but in 3% of eyes, regression had still not occurred by 3 months corrected age [79]. In the conventional treatment arm of the ETROP study, ROP regressed without reaching threshold in 33.6% of high-risk eyes. The other 66.4% underwent peripheral retinal ablation for progression to threshold [81].

146.7.2.2 Timing and Rate of Progression

The median age at onset of prethreshold ROP was 36.1 PMA in the CRYO-ROP as well as in the ETROP study [79], occurring before 32.1 weeks PMA in 5% of infants enrolled in the ETROP trial [78], and before 30.9 weeks PMA in 1% of babies in the CRYO-ROP study [78]. In the ETROP trial, 95% of babies developed prethreshold at 7 postnatal weeks or more [79] and in the CRYO-ROP study 99% of eyes were free of prethreshold ROP before 4.7 weeks postnatal age [77]. Subhani et al [77] reported earlier onset of prethreshold disease with 3.2% of babies developing prethreshold before 30 weeks PMA in the most premature infants, but the definition of prethreshold in this study differed from that used in the ETROP trial and CRYO-ROP studies.

The earliest onset of threshold ROP is reported between 31.0–32.6 weeks PMA and 6.6–8.0 weeks postnatal age [77, 80]. The earliest age of onset of stage 3 disease is reported between 30.3–35.6 weeks PMA, and 3.8–6.7 weeks postnatal age [76, 79, 82].

In the CRYO-ROP study, threshold ROP developed in 6% of infants, at a mean PMA of 37.7 weeks. The median time from prethreshold to threshold disease did not exceed 1 week [75]. Of note, aggressive posterior ROP progressing from onset to stage 4 in less than a week has also been reported.

146.7.2.3 Risk Factors for Progression and Unfavorable Outcome

Lower birth weights and younger gestational age carry an increased risk of reaching threshold ROP. Eyes in which vascularization has grown only into zone I before 35 weeks PMA have a 30% risk of developing threshold ROP compared to eyes in which vascularization have reached zone III. At fundus examination, risk factors for progression and unfavorable structural outcome include the presence of plus disease, zone I or posterior zone II ROP, increased circumferential involvement and stage 3 ROP [75]. A faster rate of progression to prethreshold disease is also associated with an unfavorable outcome [83]. Conversely, infants with zone II ROP without plus disease or with zone III ROP are at very low risk (<1%) of developing sight-threatening ROP and unfavorable outcome [78, 80, 83]. The risk to the baby's sight appears to be minimal in infants with acute ROP that demonstrated involution by moving from zone II to zone III with moderate ROP [78, 80].

146.7.3 Complications

Unfavorable visual outcome may be observed in up to 40–50% of severe ROP at one year follow-up compared to less than 1% in infants with no or less severe ROP. Prior to the introduction of retinal ablation to stop the progression of ROP, infants with prethreshold disease were predicted to have a 50% risk of blindness [74].

146.7.3.1 Anatomical Abnormalities

Apart from retinal detachment (stage 4 ROP), the main retinal and vitreal complications of ROP include retinal folds, foveal heterotopia, preretinal membranes and hemorrhages. In the CRYO-ROP study, eyes with zone I ROP or with zone II, stage 3+ were reported to have a higher risk of retinal and vitreous damage than eyes with less severe ROP [83].

Serious anatomical damage of the anterior segment can also occur in infants with severe ROP. Synechiae were observed

in 37.4% of eyes with stage 3–5 ROP, and cataract was diagnosed in 6.3% of cases in the CRYO-ROP study [83].

146.7.3.2 Refractive Errors

Twenty percent of children enrolled in the CRYO-ROP study were found to be myopic at one year of age [84]. Approximately 70% of eyes with high-risk prethreshold ROP during the neonatal period were myopic in early childhood in a follow-up report from the ETROP Study Group [85, 86]. The proportion of eyes with high myopia increased steadily between 6 months and 3 years [86]. Prevalence of myopia increased with increasing severity of cicatricial ROP, but was not related to severity of acute-phase ROP (zone of disease, presence of plus disease) [85, 86].

Prevalence of astigmatism in preterm children who participated in the ETROP Study was similar in either arm of treatment (early or conservative). By 3 years of age, astigmatism ≥ 1.00 diopters occurred in 43% of eyes treated at high-risk prethreshold ROP; 20% of eyes had astigmatism ≥ 2.00 diopters [87].

146.7.3.3 Loss of Visual Field

Visual field was shown to be reduced by approximately 30% in children with ROP, whether they underwent retinal ablation or not, compared with infants without ROP [88].

146.7.3.4 Strabismus

The prevalence of strabismus in the first year of life was 14.7% and was correlated with ROP severity in the CRYO-ROP study [89].

146.7.3.5 Late Sequelae

The percentage of eyes with unfavorable outcome increases over time. In the CRYO-ROP study, nearly half of eyes developed unfavorable outcomes at ten years [90]. Unfavorable visual outcome (vision ≤ 20/200) and unfavorable structural outcome (posterior retinal folds or worse) were reported in 62.1% and 47.9% of untreated eyes, respectively [90]. Compared to outcome at 5 years, total retinal detachments had continued to occur in control eyes, increasing from 38.6%–41.4% at ten years [90].

Although surgical treatment of severe ROP is associated with better long-term visual and structural outcomes, it still carries a risk of both short- and long-term ophthalmic morbidities, including retinal detachment and blindness. Laser-treated severe ROP remained associated with unfavorable visual outcome in 11% of cases at 3 months of age [91]. Total retinal detachment was observed in 22% of treated eyes in the CRYO-ROP 10-year follow-up study [90]. Iris atrophy and cataracts have also been reported following peripheral retinal ablation [92].

146.7.3.6 Functional Changes

While ROP is often considered to be primarily a disease of the developing retinal vasculature, the vasculopathy phase has been shown to resolve itself in some cases [93]. In contrast, despite the vascular repair, functional sequelae as revealed with the flash electroretinogram (ERG) and multifocal electroretinogram (mfERG), for example, have been shown to carry on into adulthood. In patients with a history of ROP, significant abnormalities in the a- and b-waves of the ERG have been shown to persist in the absence of any abnormal retinal vasculature [94, 95]. Furthermore, elevations of the scotopic visual thresholds in children with a history of resolved, mild ROP in addition to the slower maturation of the rod-mediated thresholds in the central retina are also suggestive of photoreceptor involvement [96–98]. Interestingly, a differential susceptibility between cone and rod function has been suggested in subjects with a history of preterm birth, where higher cone sensitivity and minimal cone dysfunction could be observed compared to that of rods [96]. While this discrepancy requires further elucidation, it has been postulated that the earlier maturation of the cones might carry out a beneficial protective effect with respect to their function in comparison with the relative immaturity of the rods that might render them more susceptible to oxidative stress [96].

Significant deficits in amplitude and implicit time of the mfERG, a central retinal response, have also been shown in subjects whose clinical features of ROP had completely resolved more than 10–20 years earlier [98]. These findings suggest that a history of ROP can carry out lasting consequences on central retinal development as revealed with the mfERG. ON- and OFF-bipolar cells contribute to this central response, and therefore amplitude attenuation might occur should ROP specifically target these cells, thereby resulting in an altered summation of depolarization and hyperpolarization in these subjects [98]. More recently, the morphologic changes of the macular structure have also been studied in formerly preterm children with mild or no sequelae of regressed ROP using optical coherence tomography (OCT). Findings in formerly preterm infants revealed a larger central retinal region in addition to a decreased foveal depression compared to children born at full term, which was suggested to result from problematic centrifugal movement of foveal cones as well as inner retinal cells throughout retinal maturation [99]. Overall, despite the resolution of vascular abnormalities that are commonly reported in ROP patients, careful consideration and monitoring of structural changes and neural dysfunction in the retina that can carry on throughout adulthood is warranted.

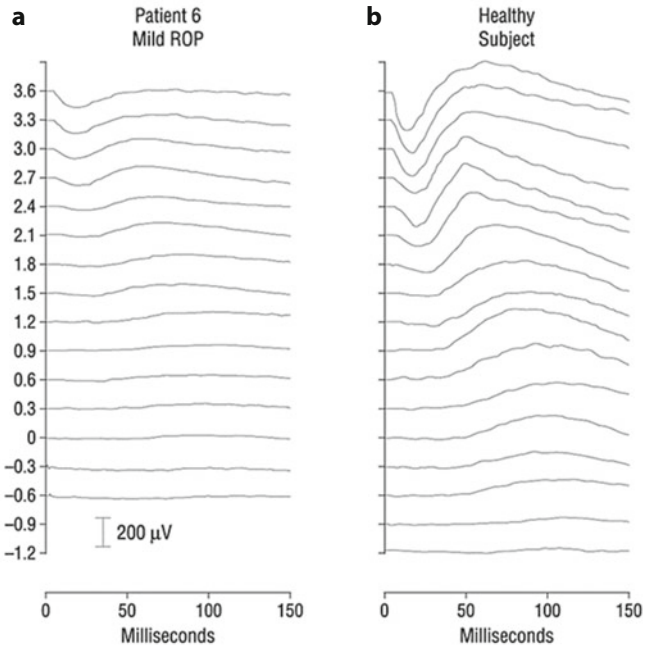

Fig. 146.6 **a** Sample electroretinographic record at 2.5 months post term in patient 6 with mild retinopathy of prematurity (ROP). **b** Electroretinographic record of a healthy, term born infant. The numbers to the left of each trace indicate the stimulus in log scotopic troland seconds. Adapted from [94]

Several other types of visual manifestations, in addition to ROP, have been thought to result from the challenges of premature birth on the developing ocular system. For example, an increased incidence in refractive errors such as myopia, astigmatism and anisometropia has been noted, in addition to an increased incidence in strabismus [100–102]. An association between the oscillatory potentials (OPs) of the ERG and ametropias has also been reported in patients with a history of ROP. For example, while a stronger ON signal and lower OFF signal (as revealed by an increase in OP_3 and a decrease in OP_4, respectively) was found in myopic patients, the reverse was documented in hyperopic patients, and thus it could be concluded that the ON and OFF pathways might play a role in determining refractive development in these patients [103]. Similar to the manifestations of ROP on eye growth and movement, visual functions including visual acuity, contrast sensitivity, color vision and visual fields may also be significantly altered and can consequently impact psychological and educational development [100, 102].

146.7.4 Surveillance of ROP and Follow-up

146.7.4.1 Screening

Based on published data regarding evolution of ROP, screening guidelines to identify infants requiring treatment for ROP while minimizing the number of stressful examinations have been established in many countries [104, 105]. In industrialized countries, they vary little, with respect to the gestational age and birth weight of screened infants, timing of first examination and frequency of subsequent examinations. Screened infants are born between 1250 and 1500 g and between 30 and 32 weeks PMA. Screening recommendations also generally include selected larger or more mature infants with unstable clinical course who are considered at high risk by the attending neonatologist [104]. In countries with low/moderate level of development, where larger and more mature infants develop severe ROP [23, 82], ROP screening programs include infants up to 1750 g and 33 weeks PMA [82]. Timing of first examination range between 4 and 6 weeks of life or 31 and 33 weeks PMA, whichever is later [82]. Subsequent follow-up examinations are recommended biweekly when examination is normal, or at least once a week in other cases. In the absence of any ROP, screening can be stopped when the retinal vascularization is completed, i.e., when retinal capillaries have grown into zone III. Screening by an experienced ophthalmologist is recommended, using binocular indirect ophthalmoscopy after pupillary dilation (Box 146.1).

As an example, the recently updated screening recommendations for ROP published jointly by the American Academy of Pediatrics (AAP), the American Academy of Ophthalmology (AAO) and the American Academy for Pediatric Ophthalmology and Strabismus (AAPOS) are summarized in Box 146.2 [104].

Telemedicine Approach to Screening for ROP

Technical advances have permitted an innovative approach to remote ROP evaluation, using digital retinal imaging systems. Theoretical advantages of this technique include, for NICUs with poor access to experienced ophthalmologists, a

Box 146.1 Pain prevention during screening for ROP

Screening examination is performed using indirect ophthalmoscopy after pupils dilation. In some cases, an eyelid speculum and a scleral depressor are used to rotate the eye in the appropriate position, making the examination stressful and painful for the baby. ROP screening examinations can have short-term effects on blood pressure, heart rate and respiratory function in the premature baby. The baby should therefore be clinically stable at the time of the examination, which should be kept as short as possible. Nesting, use of oral sucrose and use of a topical anesthetic are recommended prior to screening of babies for ROP.

BOX 146.2 Screening guidelines from the AAP/AAPOS and AAO

Infants to be screened
- infants with a birth weight < 1500 g, or
- with a gestational age < 30 weeks
- selected infants with a birth weight between 1500 and 2000 g or gestational age of more than 30 weeks with an unstable clinical course, including those requiring cardiorespiratory support and who are believed by their attending pediatrician or neonatologist to be at high risk.

Timing of initial examination

GA (wks)	Postmenstrual age at first examination (wks)	Chronologic age (wks)
22	31	9
23	31	8
24	31	7
25	31	6
26	31	5
27	31	4
28	32	4
29	33	4
30	34	4
31	35	4
32	36	4

Frequency of subsequent examinations
- 1-week or less follow-up:
  - stage 1 or 2 ROP: zone I
  - stage 3 ROP: zone II
- 1 to 2-week follow-up
  - immature vascularization: zone I – no ROP
  - stage 2 ROP: zone II
  - regressing ROP: zone I
- 2-week follow-up
  - stage 1 ROP: zone II
  - regressing ROP: zone II
- 2 to 3-week follow-up
  - immature vascularization: zone II – no ROP
  - stage 1 or 2 ROP: zone III
  - regressing ROP: zone III

Termination of screening criteria

a. zone III retinal vascularization attained without previous zone I or II ROP (if there is examiner doubt about the zone or if the postmenstrual age is less than 35 weeks, confirmatory examinations may be warranted)
b. full retinal vascularization
c. postmenstrual age of 45 weeks and no prethreshold disease (defined as stage 3 ROP in zone II, any ROP in zone I) or worse ROP is present; or
d. regression of ROP (care must be taken to be sure that there is no abnormal vascular tissue present that is capable of reactivation and progression).

decrease in the number of eye examinations that would require an evaluation by an ophthalmologist. Although recent studies have shown promising results [106], evidence base for digital imaging remains scarce, and clinical examinations by an experienced ophthalmologist remains the standard for identifying ROP [107].

146.7.4.2 Ophthalmologic Follow-up Beyond the Neonatal Period

Although the risk of long-term visual disorders, such as refractive errors, strabismus or amblyopia is well recognized in ROP, there is little consensus regarding when, how frequent and forhow long ophthalmologic follow-up should take place [108]. Eye examination is indicated after discharge [104], even in case of ROP regression. An early first post-discharge examination, for example within 6 months of expected full-term birth, could help detect amblyogenic factors and reduce the risk of later visual impairment.

146.8 Treatment

146.8.1 Cryotheraphy

Cryotherapy to the vascular peripheral retina of eyes with severe forms of active ROP had been used in Japan since the early 1970s [109] and gradually gained advocates in several countries. Many ophthalmologists were reluctant to employ this treatment modality because of untoward or unexpected results. In the CRYO-ROP study, the method used to ablate

avascular retina was cryotherapy. This treatment is still used in some hospitals, but it largely has been supplanted by diode laser in many countries. The cryotherapy probe is a long, thin, curved metal hand piece with a slightly rounded tip. It is applied to the external eyeball transconjunctival or trans-scleral in forms more posterior using the indent of the wall of the eye. This indentation can be seen using the indirect ophthalmoscope. A foot pedal attached to the probe is depressed when it is seen to be in proper position. The retina blanches as it freezes, and leaves a treated spot. The probe is removed and moved to the next spot, either contiguous with or leaving a small space next to the previous treatment. The process is repeated over and over until the entire unvascularized retina has been treated. Cryotherapy is a painful and inflammatory procedure and needs general anesthesia. Adverse effects include conjunctivitis, eyelid swelling, hypotony, infection, retinal detachment, and glaucoma. Pressing on the eye with the cryoprobe may elicit the oculocardiac reflex, a sudden bradycardia. Infants are usually put on topical antibiotics and steroids for a week or so after cryotherapy [70].

146.8.2 Photocoagulation

The treatment of ROP has evolved significantly since the advent of the CRYO-ROP study, the trial that proved the benefit of ablating the peripheral avascular retina in ROP [110]. A fifteen year follow-up of this study, published in 2005, describes a decrease of over 40% and 30% in unfavorable structural and visual outcomes respectively, in treated versus observed eyes [110]. Easier to administrate, and with less adverse effects than cryotherapy, laser photocoagulation soon became the modality of choice to treat ROP. The treatment ablates the peripheral avascular retina (Fig. 146.7), which causes the stimulus for neovascularisation to decrease, probably through decline of angiogenic factors. Many studies demonstrated that laser was at least as effective as cryotherapy in preventing unfavorable outcomes. The use of cryotherapy is now restricted to particular cases in which fundus visualization is poor (i.e., vitreous hemorrhage), thus preventing laser administration.

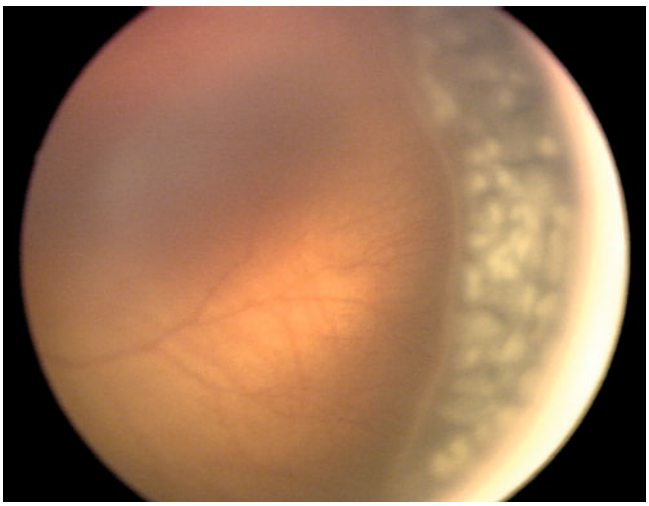

Fig. 146.7 Diode laser photocoagulation of the peripheral avascular retina

Table 146.2 Treatment strategies based on stage and location (zone) of ROP

Consider early treatment of eyes with pre-threshold-type 1 disease: – Zone 1, any stage of ROP with plus disease – Zone 1, stage 3 with or without plus disease or – Zone 2, stage 2 or 3 with plus disease
Observe closely eyes with pre-threshold-type 2 disease (Consider treating when progression to type 1 or threshold ROP): – Zone 1, stage 1 or 2 with no plus disease or – Zone 2, stage 3 with no plus disease

Laser photocoagulation was used for years following the CRYO-ROP recommendation: to treat threshold ROP, a point in the progression of the disease at which the risk of retinal detachment approached 50% [110]. Nonetheless, poor visual results remained common with such treatment criteria. The CRYO-ROP study demonstrated at 10 year follow-up that 44.4% of eyes with severe ROP had vision of 20/200 or worse [111]. The need for earlier intervention became evident and the Early Treatment for ROP study (ETROP), published in 2003, provides our current treatment guidelines (Table 146.2) [75]. In that trial, infants with high-risk pre-threshold ROP had one eye randomized to early intervention and the contralateral-control eye to conventional treatment. Unfavorable visual and structural outcomes were reduced from 19.8% to 14.3% and 15.6% to 9%, respectively. Some recent laser treatment long-term outcome studies report best mean corrected visual acuities between 20/46 and 20/98 [112–114]. Over 65% of the eyes from those series had vision of 20/40 or better.

Laser photocoagulation, applied transpupillary through an indirect ophthalmoscope can be performed in the nursery or operating room. Argon laser was used initially but is now widely replaced by Diode laser, which requires less power. Many studies described placement of laser burns 0.5–1 burn width apart. We favor a more confluent pattern of treatment. Laser burns were applied in the area of the avascular retina extending from the ridge of extraretinal fibrovascular proliferation to the ora serrata for 360°. More continous treatment may not only reduce the rate of progression [115, 116] but also the need for retreatment [115]. Generally, regression can be seen 5–7 days after treatment. Retreatment must be considered when retinopathy continues to progress and there are skipped areas that can be treated.

Laser treatment needs topical anesthetic and intravenous sedation in spontaneous respiration. Complications from indirect laser photocoagulation include cataract, corneal or iris burns, choroidal or vitreous hemorrhage, hyphema, choroidal

rupture and phthisis bulbi [117]. Reduction of the visual field is minimal and not felt to impact visual function [113].

The refractive status of treated eyes deserves special mention, since myopia remains a significant and very common finding in all series of patients treated with laser. In a randomized bilateral treatment study, Connolly et al [118] found that laser treated eyes were less myopic than those treated with cryotherapy (mean spherical equivalent –4.48 and –7.65 diopters, respectively). Other authors have found similar results [119, 120]. The problem of myopia is compounded by the fact that prematurity, ROP itself and the treatment predispose to this refractive error [84, 121–123].

146.8.3 Anti-VEGF

Anti-VEGF treatment is reserved for premature infants affected by AP-ROP.

VEGF is a dominant factor that contributes to the neovascular phase of ROP. Accordingly the potential use of anti-VEGF therapy during this phase has been proposed. bevacizumab is a monoclonal antibody against VEGF that has been approved for use in colorectal, breast and lung cancer; this antibody has also been widely used for proliferative macular degeneration and diabetic retinopathy. Since the cryotherapy and early treatment ROP trials detected an unfavorable visual outcome in relatively advanced proliferative retinopathy in 78% and 55% of infants respectively, it was reasonable for practitioners to attempt using anti-VEGF therapy with the hope of improving outcome. Until 2009, nine reports (including 6 case reports, 2 retrospective studies, and 1 prospective study) had documented results of anti-VEGF therapy in ROP [124]. A generally favorable outcome (controlled progressive neovascularization) was detected in 6 of the 9 small reports [125–130] (Table 146.3). However, timing (stage/zone of ROP), dose (range: 0.4–12.5 mg iv), and frequency of administration of bevacizumab, as well as co-treatment with photocoagulation, varied tremendously between reports. Recently, in a multi-center randomized prospective trial patients treated with bevacizumad had significantly less recurrence of neovascularisation requiring treatment than eyes treated with diode laser for zone 1, stage 3 ROP plus. No statistical difference between the two treatment modalities was identified for zone 2, stage 3 ROP plus [131]. The interpretation of the results of the trial has not escaped controversy [132]. The unknown possible deleterious effects that blocking VEGF may have on the developing brain constitutes the principal concern over the incorporation of a new promising treatment for ROP [133]. A number of issues need to be addressed in future well-conducted randomized trials: a) adjunct photocoagulation therapy with anti-VEGF treatment; b) impact on retinal ganglion cell integrity, since these neurons express the receptor for VEGF and are cytoprotected by VEGF; c) timing of anti-VEGF therapy; d) systemic effects of larger doses (1.25 mg) of bevacizumab, notably on cerebral vasculature; e) long-term impact on visual acuity and visual fields; f) cost-benefit versus currently available treatment.

146.8.4 Scleral Buckling and Vitrectomy

Almost one in 10 infants will develop unfavorable structural outcomes despite appropriate laser treatment [123]. Stages 4 and 5 of ROP will often require surgical treatment. It is however, possible to observe a small, localized and shallow retinal detachment which may not progress or can even reattach, especially in the absence of active neovascularization and plus disease [134]. Examinations and treatment decisions must be done by an experienced ophthalmologist.

Acute ROP related retinal detachments result from contraction of neovascularization along the ridge and growth of vessels into the overlying vitreous leading to traction on the retina. Exudation causing subretinal accumulation of fluid is a much less common mechanism. Available techniques to treat retinal detachments from ROP include scleral buckling and different modalities of vitrectomy. The latter has become more frequently utilized by vitreoretinal surgeons and in a comparative nonrandomized study Hartnett et al found it superior to scleral buckling to treat stage 4 retinal detachment [135]. The choice between the two procedures is, nonetheless, controversial and scleral buckling is still used when the retinal traction is located near the equator of the eye or anterior to it [134, 136, 137].

Scleral buckling (encircling the eye at the equator or anteriorly with a silicone band) intends to correct the retinal detachment mechanically by moving the wall of the eye closer to the detached retina, thus counteracting the forces that exert traction on it. In a recent study using fundus fluorescein angiography Yokoi et al [138] have suggested that scleral buckling may also reduce the neovascular activity in eyes with peripheral proliferation. They speculated that retinal reattachment may improve the vascular supply from the choroid to the retina, therefore decreasing the stimulus for VEGF expression. Buckling is not useful to treat posterior detachments in zone 1 or posterior zone 2, in which the ridge is located behind the equator and there is a large anteriorly attached retina. Buckling has a success rate of retinal reattachment in stage 4A ROP of around 70% [139]. A major disadvantage of buckling is that the indentation caused by the silicone band elongates the eye inducing myopia of up to –9 or –11 dioptres [140, 141], thus increasing the risk of amblyopia, a significant concern given the age group. Furthermore, a second operation is required later to divide or remove the buckle to promote normal growth of the eye, improve its anterior circulation and correct the refractive error.

Vitrectomy works through direct release of vitreoretinal traction forces. The removal of angiogenic factors like VEGF and vasodilators during vitrectomy may constitute an additional mechanism of action [137]. In the past it was necessary

Table 146.3 Case reports on use of bevacizumab for severe ROP

Authors, Year	No. of patients (eyes treated with bevacizumab)	GA (wks)	BW (g)	State before use of bevacizumab (as described)	Previous therapy for bevacizumab-treated eye(s)?	Treatment use in conjunction with bevacizumab	Dose of bevacizumab used (mg)	Favorable outcome with bevacizumab?
Chung et al, 2007 [125]	1 (2)	25	884	Zone I, stage 3+	No	Laser photocoagulation	0.75	Yes
Honda et al, 2008 [126]	1 (1)	23	598	Zone I, stage 4A+	Yes	None	Right eye, 0.4	No
Kong et al, 2008 [127]	1 (2)	22	350	Zone I, stage 2+ Zone II, stage 3+	No No	None None	0.5 0.5	Yes Yes
Shah et al, 2007 [128]	1 (1)	31	1170	Anterior NV after laser for AP-ROP	Yes	None	Left eye, 0.75	Yes
Lalwani et al, 2008 [129]	3 (5)	23	600	Threshold ROP Reactivation of NV	No Yes	None Laser 1 wk prior	1.25 Right eye, 0.63	Yes Yes
		24	760	Zone 1 plus disease	No	Laser photocoagulation	Right eye, 0.63	Yes
		25	835	Bilateral RD Persistent exudation Vascular engorgement	Yes Yes No	None Laser photocoagulation None	Left eye, 0.63 Right eye, 0.63 Left eye, 0.63	NS No Yes
Travassos et al, 2007 [130]	3 (3)	25	510	Anterior segment involvement in posterior zone II; prethreshold	No	None	Right eye, 0.75	Yes
		25	650	Anterior segment involvement in posterior zone II; prethreshold	No	None	Right eye, 0.75	Yes
		23	649	Anterior NV after laser for zone I; prethreshold	Yes	None	Left eye, 0.75	Yes

AP-ROP aggressive posterior retinopathy or prematurity, *NS* not stated, *NV* neovascularization, *RD* retinal detachment.
Favorable outcome is defined as regression of the disease as described in each report. RD or worsening of the condition is categorized as unfavorable.
Reproduced from [24], with permission

to remove the lens in order to access the vitreous cavity. Nowadays lens-sparing vitrectomy has become the preferred technique of many vitreoretinal surgeons to treat stage 4 ROP [131]. Early vitreous surgery is advised when the laser fails and the signs of progression are evident: vitreous torbidity, pupillary rigidity, persistent plus disease. The recently introduced vitrectomy 25 G, is an approach suited to the small eye of premature baby. The eye is entered through the pars plicata and the procedure allows treatment of retinal detachments resulting from the posterior zone of ROP, in which operating posterior to the equator is necessary [134]. When more anterior disease causes retrolental membranes, removal of the lens may be necessary (vitrectomy and lensectomy) [131]. The response to vitrectomy is unfavorable when done in eyes with active neovascularization. Unfortunately sometimes a retinal detachment can progress rapidly in an eye that is still vascularly active; bleeding, continued proliferation and contraction can occur with the surgery [134, 135]. Other complications of lens sparing vitrectomy are cataract, infection and retinal breaks [135]. Combining buckling was not found to add any benefit to lens-sparing vitrectomy [137]. Capone and Trese found reattachment of the retina in 90% of the eyes with Stage 4A ROP treated with lens-sparing vitrectomy [142].

Stage 5 ROP is more often treated with vitrectomy. The results are, however, poor. Cusick et al reported reattachment of the retina in 28% of their cases [143]. The use of coadjuvant intravitreal triamcinolone [144] and more recently autologous plasmin [145] has been reported to improve the anatomical results of vitrectomy for ROP stage 5. No data was presented on vision in those studies.

Despite improved anatomical results over the past years, there remains an important disparity between structural and visual outcomes of surgery for ROP related retinal detachment. Poor vision is common in eyes with affected maculas (stage 4A) and stage 5. Cusick et al found vision better than 5/200 in only 8 of 183 eyes treated for Stage 5 ROP [143]. Among 12 eyes from the ETROP treated for Stage 5, 6 had no light perception, 4 had light perception only and 2 eyes had detection only of the low vision card [146]. Unfortunately, poor visual outcome occurs in less severe retinal detachments as well; in the ETROP study, only 21% of eyes treated for stage 4A maintained normal acuity 9 months after surgical repair [146]. Even though the trial may have involved eyes with inherently more severe disease, its results show that further investigation is still needed in order to improve the modest successes of surgery for ROP retinal detachment. The

use of anti-VEGF drugs may show promise in decreasing the progression of ROP to retinal detachment and is currently being investigated at some centers. In the mean time the surgical developments are likely to continue evolving.

146.8.5 Prevention

The development of preventive and less destructive therapies for ROP such as nutritional supplements, including forms of vitamins E and C, and omega 3 fatty acids, would be much more desirable, however, despite numerous trials, prophylactic supplementation with these components remains controversial; limitation of oxygenation has already been addressed above as an effective means of reducing ROP incidence and will not be repeated in this section.

146.8.5.1 Vitamin E

The most extensively studied medical treatment of ROP is the use of antioxidant vitamin E (alpha tocopherol). Vitamin E is a naturally occurring potent, free radical scavenger that decreases lipid peroxidation and helps maintain membrane integrity [147]. The retinal cells of the premature infant who has these clinical complications have their antioxidant defense system severely compromised, and they may be particularly vulnerable to the deleterious effects of oxygen derived free radicals. It is known that premature infants are born with only 10% of adult levels of retinal vitamin E [148]. The rationale for using vitamin E as a treatment for ROP stems from the idea that peroxidative damage to cells is a causal factor and that vitamin E supplements may prevent this damage. The first reports of its use in the 1940s [149] provide encouraging preliminary results of possible benefits of vitamin E prophylaxis in preventing ROP. However, this study was soon followed by the observation that oxygen therapy had a close link with ROP [150, 151], and investigations into the effect of vitamin E were abandoned until the 1970s. In 1974 Johnson et al [152] reported a randomized clinical trial using oral and parenteral alpha tocopherol acetate supplements to achieve physiological serum levels (1–3 mg/dL) in premature infants. Subsequent work showed a beneficial effect on incidence and severity of ROP associated with vitamin E prophylaxis that yielded physiologic serum levels of the antioxidants [13]. Three clinical trials enrolling 418 infants (less than or equal to 1500 g birth weight) elucidated the efficacy of vitamin E in suppressing the development of severe ROP. In this study only continuous vitamin E supplementation to adult physiologic levels from the first hours of life suppressed the development of severe ROP [153]. Other randomized clinical trials with increasing serum concentrations of vitamin E at a physiologic range, reduced by 52% the incidence of severe threshold (Stage 3+) ROP in the very low birth weight infant [154]. Despite several clinical trials showing the effectiveness of vitamin E in preventing ROP, prophylactic supplementation with vitamin E remains controversial [155]. Subsequent trials have shown no effect and questions revolve around the route of administration (oral versus intramuscular versus intravenous), dose, timing of therapy, appropriate plasma levels, and trends towards higher plasma tocopherol levels in control groups [156, 157]. The protection mechanism of vitamin E in suppressing the development of severe ROP is not well known. A report proposed that spindle cells, mesenchymal precursors of the inner retinal capillaries, are the primary inducers of the neovascularization associated with ROP and the exposure of spindle cells to elevated oxygen tension increases their gap junction area. This early morphologic event immediately halts the normal vasoformative process and eventually triggers the neovascularization that is observed clinically 8–12 weeks later. Vitamin E supplementation above the deficient plasma levels of these infants suppresses gap junction formation and clinically reduces the severity without altering the total incidence of ROP [158].

146.8.5.2 Vitamin C

Vitamin C (ascorbic acid) is an important aqueous phase antioxidant in cells and plasma [159]. Vitamin C has a number of important metabolic functions and is actively transported across the placenta [160]. Vitamin C concentrations in cord plasma are higher than the mother's and, in term infants, plasma concentrations fall considerably over the first 24 hours of life [161]. Preterm infants generally have higher cord Vitamin C concentrations than term infants, and concentrations then decline over a few days [162]. Most preterm infants receive vitamin C as part of a multivitamin supplement, but there are few data on which to base optimum concentrations [163]. One recommendation derives from the concentrations found in healthy breast fed term infants, with an adequate vitamin C concentration stated at approximately 34 mmol/L [164]. Breast milk contains 3.5–5.5 mg vitamin C per 100 mL [165, 166] so that an average infant having 150 mL/kg/day of milk will receive 5.2–8 mg/kg/day. The alternative view is that preterm infants should receive higher doses of vitamin C (25–31 mg/kg/day), to achieve concentrations closer to those in utero in the third trimester [164].

Vitamin C also has shown to have pro-oxidant activity [167, 168], and the few studies of the relation between vitamin C concentration and morbidity in very preterm infants remain controversial. Silvers et al [169] reported that plasma vitamin C concentrations within 2 hours of birth were significantly higher in infants who died compared with survivors. These researchers also observed that higher vitamin C concentrations on day 2 were associated with a greater risk of developing bronchopulmonary dysplasia [170]. In contrast, Moison et al [171] reported lower plasma vitamin C concentrations on day 10 in preterm infants who developed

bronchopulmonary dysplasia compared with those who did not. In a pilot observational study of very low birth weight infants, an increased risk of retinopathy of prematurity (ROP) with higher plasma vitamin C concentrations at day 7 and an increased risk of bronchopulmonary dysplasia with lower concentrations at 28 days was found [172]. In another study, Darlow et al [14] hypothesized that maintaining a lower plasma vitamin C concentration (target 35–50 mmol/L) in the first week of life and a higher concentration (target 90 mmol/L) in weeks 3–4 would be accompanied by improved clinical outcome and less morbidity (chronic lung disease and ROP) in very low birth weight infants.

146.9 Future Directions

Some advances have been made in reducing severe retinal damage due to aberrant pre-retinal neovascularization with the use of cryo- and photocoagulation therapy. Likewise, preventive measures to diminish adverse consequences of relative hyperoxia have also started to be tackled by reducing oxygen administration to premature infants. Yet, a number of issues require future investigations. These can be divided along the following lines: genetic predisposition; improved inhibition of pre-retinal neovascularization; and most importantly, preservation of retinal microvasculature (and parenchyma).

146.9.1 Genetic Predisposition

Despite the widespread exposure of premature infants to adverse risk factors, notably hyperoxia, relatively few babies develop severe forms of ROP; this infers genetic predisposition to advanced forms of ROP. So far only the Norrin gene and Frizzled-4 have been clearly associated with an increased risk for ROP [10, 173]. There is a need to identify other genetic susceptibility determinants.

146.9.2 Inhibition of Pre-retinal Neovascularization

In addition to anti-VEGF therapy, other solutions to neovascularization can be envisaged to affect vascular development and factors that trigger expression of angiogenic factors; along these lines, the role of anti-inflammatory agents and modulators of carbohydrate metabolite receptors [174], are also worth exploring.

146.9.3 Preservation of Retinal Microvasculature

Finally, it is paramount to preserve retinal microvasculature since its integrity prevents not only aberrant pre-retinal neovascularization but also retinal function. Hence, strategies suggested above regarding growth infer that IGF-1 may be an interesting potential avenue to pursue. Along the same lines, countering pro-inflammatory lipids using omega-3 fatty acids may also turn out to be effective [175]. In addition, acceleration of normal revascularization may also be a worthy approach. Finally, prevention of prematurity is a long-term goal.

Acknowledgments

We thank to Drs. Maria Ana Martinez, Daniel Moreno and Hugo Quiroz of the Association to Prevent Blindness in Mexico (APEC) for the contribution of the pictures in figures 146.3 and 146.4.

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Neonatal Orthopedic Surgery

Peter D. Pizzutillo and Martin J. Herman

147.1 Introduction

The physical examination of the neonate is unique and reflects stages of development. While the examination may suggest deformities of the spine and extremities, it is important to recognize physiologic variations of anatomy and growth that are characteristic of the neonate and distinguish them from pathologic anatomy. Timely recognition of clinical problems is especially important for prompt and appropriate interventions in this population. Delay in treatment of acute problems may interfere with normal limb growth and function or survival of the neonate.

147.2 Clinical Findings

While congenital anomalies of the spine or limb may not require urgent attention, they are associated with a host of anomalies involving other organ systems, such as renal, cardiovascular or spinal cord, which may require immediate evaluation and interventions. Fractures and infections are the most common clinical problems that demand early diagnosis and treatment.

In the absence of bone dysplasias, such as osteogenesis imperfecta, vaginal delivery or difficult cesarean section are associated with fractures of the clavicle, humerus, femur and injury of the cervical spine. When the infant presents with a flail upper limb, fracture of the clavicle, fracture of the proximal or distal humeral physes, brachial plexus injury or septic arthritis of the shoulder must be considered. Radiographs of the injured area may or may not confirm injury due to skeletal immaturity and incomplete ossification. Ultrasonographic evaluation of the proximal or distal humerus or proximal femur may document pathologic motion of the unossified epiphysis to confirm the diagnosis of a transphyseal fracture. Radiographs of long bones may reveal periosteal reaction that is a normal finding in 35% of infants older than 1 month and younger than 4 months of age. The periosteal reaction is characterized as regular and less than 2 mm in thickness. In the neonate, a periosteal reaction thicker than 2 mm may be associated with infection, syphilis, prostaglandin infusion, ECMO or Caffey disease. In infants older than 4 months of age, child abuse must be considered.

When more than one limb is flaccid, neonatal cervical spinal cord injury may be responsible. Injury of the cord may occur with distraction forces applied to the head. Due to the differential in plasticity of the vertebral column and the spinal cord, neurologic injury may occur without injury to the vertebral column. Radiographs of the cervical spine may appear normal and MRI would be required to diagnose injury of the cord. Complete or incomplete injury of the cervical spinal cord may present as a stillborn infant or an infant with significant neurologic deficits and delays that suggest the diagnosis of cerebral palsy. Early diagnosis is important since incomplete injury of the cord requires immobilization of the spine to prevent increasing injury.

Infection of the musculoskeletal system is of special concern in the neonate since the immature immune system may not express the expected findings of fever or abnormal laboratory studies, such as white blood cell count, ESR and CRP. Early examination of an infected joint may not reveal any signs of inflammation and may create a false sense of security that will delay diagnosis. A more common presentation of infection in the neonate includes diminished interest in feeding, irritability and decreased motion of an affected limb. Common sites of infection involve the hip, knee and shoulder joints with associated osteomyelitis. Ultrasonographic evaluation of suspected joints will confirm the presence of fluid and can guide aspiration of the joint. MRI of an infected joint is helpful to determine the coexistence of osteomyelitis. Multiple sites of infection are the rule in the neonate and effective treatment includes not only appropriate antibiotic coverage of

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Table 147.1 Common musculoskeletal findings and diagnoses

Neck	Head tilted with contralateral rotation, mass in sternocleidomastoid muscle Limited neck motion, short neck (Klippel-Feil syndrome)
Arm	Swelling, tenderness clavicle (fracture) Flail arm (brachial plexus injury) Swelling, diminished movement (infection, fracture) Radial deviation of hand (TAR syndrome) Polydactyly, syndactyly
Spine	Curvature (scoliosis) Rib abnormalities (congenital scoliosis, spondylocostal dysplasia) Midline defects (myelomeningocele, spinal cord anomalies)
Hip	Positive Ortolani/Barlow (DDH) Short femur (DDH, congenital anomalies of the femur, skeletal dysplasia)
Leg	Tibial bowing Posterior apex (calcaneovalgus foot) Anterolateral apex (congenital pseudoarthrosis of tibia, fibular hemimelia) Hyperextended knee (congenital knee dislocation)
Foot	Medial midfoot crease, forefoot inward (metatarsus adductus) Fixed ankle equinus with forefoot down and inward, heel inward (clubfoot) Rocker bottom foot (congenital vertical talus) Foot dorsiflexed and touching shin (calcaneovalgus foot)

Table 147.2 Common traumatic birth injuries and their associated physical findings

Fractures	
– Clavicle	Tenderness and swelling over mid-clavicle, diminished spontaneous arm movement (pseudoparalysis) Consider congenital pseudarthrosis if right-sided
– Humerus	Upper arm swelling, crepitus, diminished spontaneous arm movement (pseudoparalysis)
– Elbow	Elbow swelling, deformity, painful and limited elbow ROM
– Femur	Thigh swelling, pain with diaper changes, limited hip ROM
Brachial plexus	
– Erb's palsy	Shoulder internal rotation, elbow extension, wrist flexion Diminished spontaneous arm movement proximal to hand
– Complete plexus injury	Flail arm without spontaneous movement Ptosis of the ipsilateral eye (Horner's syndrome)
Spinal cord injury	
– Apnea	
– No spontaneous bilateral extremity movement	
– Progressive increase in extremity tone with truncal hypotonia	
Cephalohematoma	
– Focal area of head swelling, ecchymosis	

Table 147.3 Important orthopedic diagnoses and syndromes in the NICU

Achondroplasia
- Frontal bossing, depressed nasal bridge, midface hypoplasia
- Global hypotonia
- Joint laxity
- Apnea, foramen magnum stenosis
- Thoracolumbar kyphosis
- Hip flexion contracture
- Rhizomelic short limbs

Spondylocostal Dysplasia (Jarcot-Levin syndrome)
- Prominent occiput
- Short neck
- Thoracic hypoplasia and insufficiency
- Chest wall defects, rib fusions
- Congenital scoliosis

Down syndrome
- Depressed nasal bridge, medial epicanthal folds, hypertelorism
- Simian crease
- Global hypotonia
- Joint hyperlaxity
- Hip dysplasia

Arthrogryposis
- Severe joint stiffness
- Elbow extension, wrist flexed and in ulnar deviation, fingers flexed with thumb in palm deformity
- No skin creases in antecubital fossa or palm
- Hip and knee flexion contractures
- Hip dislocations
- Severe, rigid clubfeet

Myelomeningocele
- Midline low thoracic or lumbar spine skin defects with exposed meninges
- Limited or no spontaneous movement of lower extremities
- Exaggerated reflex response in lower extremities
- Hip dislocation
- Clubfeet or congenital vertical talus

Caffey's Disease
- Diminished spontaneous arm movement (pseudoparalysis)
- Swelling of mandible or extremity, especially the ulna
- Exuberant periosteal reaction by radiographs
- Evaluation inconsistent with infection or trauma

Table 147.4 Hallmarks of musculoskeletal infection in the newborn

- Irritability, poor feeding, focal limb swelling and diminished spontaneous limb movement (pseudoparalysis)
- Most common sites: hip, knee, and shoulder
- Most common diagnoses: osteoarticular infections and cellulitis with soft tissue abscesses of the extremities
- Most common infectious agents: *Staphylococcus aureus* and gram negative bacteria
- Most common complication: joint and angular deformities and leg-length inequality

Pitfalls
1. Immature immune response may delay diagnosis and treatment
2. Osteomyelitis and septic arthritis often occur concurrently and at multiple sites
3. Bone scan is less sensitive than other imaging modalities for identifying infection; ultrasound and MRI most useful

Staphylococcus aureus and Gram negative organisms but also surgical drainage of septic joints. Repeated daily aspiration of the septic hip joint is not capable of complete evacuation of harmful inflammatory elements and is painful. Repeated aspiration of the knee or ankle may be effective, but surgical drainage of the involved hip joint is the standard of care.

147.3 Diagnosis and Syndromes

Delay in diagnosis and treatment of septic arthritis of the hip results in impaired physeal function, progressive angular deformity of the limb, limb length discrepancy and permanent destruction of the hip joint.

Developmental dysplasia of the hip (DDH) joint responds well to nonoperative intervention when treatment is initiated in infancy. Fixed dislocation of the hip, the so-called teratologic dislocation, is rare and associated with more generalized disorders, such as arthrogryposis and Larsen syndrome. DDH is associated with breech presentation and delivery, congenital muscular torticollis, foot deformities and congenital dislocation of the knee.

There are a host of musculoskeletal conditions that may be diagnosed in the neonate. Tables 147.1–147.4 provide a menu of commonly occurring problems. A high index of suspicion for occult acute clinical problems will serve the clinician well in the care of these unique patients. Specific information of each of these conditions may be readily found in textbooks of pediatric orthopedics.

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Neonatal Skin Disorders

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148.1 Neonatal Skin Structure and Functions

By the end of pregnancy, fetal skin anatomy and composition are similar to those of adults. By 22–24 weeks all anatomical elements are present, while functional and biochemical maturity takes several years to achieve (Table 148.1). There are structural differences between the skin of the preterm neonate, term neonate and adult that have significant physiological and clinical consequences [1].

The primary functions of neonatal skin are mechanical protection, thermoregulation, prevention of insensible fluid loss and immune surveillance. The skin of premature infants is thinner and much less effective in these functions. It is subject to fissuring from use of adhesives and their removal. Chemical burns from topical alcohol swabs or disinfectants, and thermal burns from transcutaneous oxygen monitoring are well-recognized problems in neonatal intensive care. Neonatal skin has increased permeability that reflects the reduced water-holding capacity of the stratum corneum. Transcutaneous absorption is increased in neonates, especially premature infants, and may lead to local and systemic side effects from topical agents such as iodine soap, hexachlorophene, benzyl alcohol and steroids (Table 148.2 and Fig. 148.1) [2]. The increased permeability of neonatal skin can also be an alternative method of drug administration. Other structural parameters that affect neonatal skin permeability are low surface lipid production, epidermal and dermal thickness due to incomplete development, and an under-developed basement membrane and papillar vasculature. Altered permeability of neonatal skin also permits transepidermal water loss from the internal environment, which can lead to extreme dehydration, and poor autonomic control of skin vessels and reduced sweating contribute to poor thermal regulation in preterm infants.

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148.2 The Physical Dermatological Examination of the Newborn

It is important in the newborn, to evaluate the total skin surface including the adnexa, the oral mucosa and the genitoanal region. Dermatological examination is primarily morphologically and descriptively oriented. A good knowledge of elementary skin lesions is essential for a reliable description of dermatoses (Table 148.3).

148.3 Transient Physiologic Disorders in the Newborn

Normal physiological characteristics of newborns that reflect immaturity and transition from life in utero should be recognized and differentiated from true disease.

148.3.1 Vernix Caseosa (VC)

VC is a white waxy or cheese-like substance coating the skin of newborns. It is composed of sebum, shed lanugo and sloughed off fetal skin cells. VC is secreted by sebaceous glands around the 20th week of gestation, and decreases in amount toward the end of gestation. It is thought to protect the baby's skin from dehydration in the womb. Vernix colour and odour can reflect intrauterine problems as in hemolytic diseases (yellow-brown discoloration) or infections.

148.3.2 Rubor and Acrocyanosis (RaA)

RaA are manifestations of vasomotor instability, frequent in neonatal life. Generalized redness, reflecting vasodilatation,

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Table 148.1 Structural differences between preterm neonate, term neonate and adult skin

	Adult	Term infant	Preterm infant (30–32 weeks)
Skin surface	Dry	Vernix	Vernix (gelatinous)
Skin thickness	2.1 mm (media)	1.2 mm	0.9 mm
Epidermal thickness	50 μm	50 μm	27.4 μm
Stratum corneum thickness	9–15 μm	9–10 μm	4–5 μm
Melanocytes	Number decreases with ageing	Number as in young adults, fewer melanosomes and low melanin production	Number as in term infants, few mature melanosomes
Dermal epidermal junction	Ridged; Antigen expressed	Flat but ridged; Antigen expressed	Flat but ridged; Antigen expressed
Anchoring filaments, fibrils and hemidesmosomes	All normal	All normal	All fewer and smaller
Papillary dermal collagen	Normal	Normal	Edematous
Reticular dermal collagen	Normal	Smaller-intermediate boundless with the hypodermis	Much smaller boundless with the hypodermis
Reticular dermal elastic fibers	Normal	Smaller in size and immature in structure	Finer than in term infants, immature in structure
Reticular dermal cell count	Fibroblasts +	Fibroblasts ++	Fibroblasts +++
Hypodermis	Well developed	Well developed	Well developed

Table 148.2 Potential dermatological and/or side/untoward effects from topical application of some skin care applied substances in newborns

Agent	Vehicle/Use	Neurologic side/Untoward effect(s)	Dermatologic side/Untoward effect(s)
Adhesives	Adhesives removal	–	Toxic epidermal necrolysis
Adhesive monitoring leads	Adhesives devices	–	Anethoderma of prematurity, skin breakdown
Alcohols	Topical antiseptic	–	Skin necrosis (expecially in immature occluded skin)
Boric acid	Diaper powder	Seizures	Erythrodermia
Corticosteroids	Topical anti-inflammatory agent	–	Skin atrophy, striae distensae
Glycerin	Emollient	Seizures	–
Hexachlorophene	Antiseptic cleanser	Vacuolar encephalopathy, seizures	–
Lidocaine	Local anesthetic	Seizures	–
Lindane	Scabicide lotion	Seizures	Rash
Neomicin	Local antibiotic	Ototoxicity, neural deafness	Conctact dermatitis
Pentachlorophenol	Laundry detergent	–	Sweating
Propylene glycol	Excipient	Seizures	–
Salicylates	Keratolytic ointment	Encephalopathy	–
Silver sufadiazine	Topical antibacterial	Kernicterus	Argyria
Tincture of benzoine	Antiseptic	–	Skin breakdown
Triclosan	Topical antibacterial	–	Conctact dermatitis

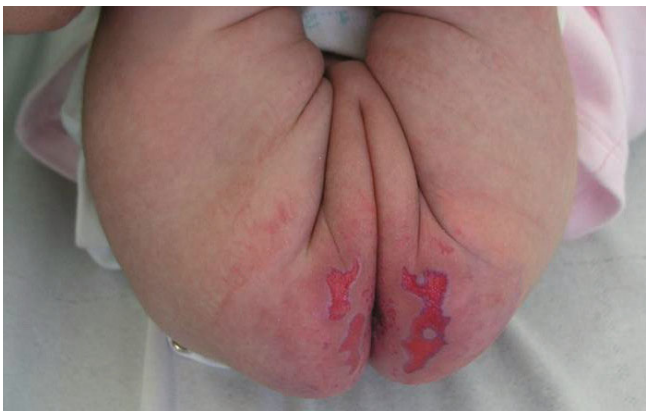

Fig. 148.1 Severe acute irritant dermatitis in a newborn, with erosive lesions of the perianal zone

is often present at the birth, associated with peripheral acrocyanosis. Acrocyanosis has no pathologic significance and gradually disappears after several weeks. It presents as bilateral, symmetric, intermittent bluish discoloration of the hands and feet.

148.3.3 Cutis Marmorata (CM)

CM is a transient benign reticulate bluish discoloration of the skin occurring in preterm and full-term infants, due to an exaggerated vasomotor response to hypothermic stress. It disappears after warming the infant and resolves after a few weeks. Profound or persistent CM has been reported in

Table 148.3 Lesions in dermatological semeiology

Type	Description or definition
Erythema (P)	Redness of the skin caused by dilatation and congestion of the capillaries
Wheal (P)	Skin elevation caused by swelling that can be itchy and usually disappears soon after erupting
Papule (P)	Circumscribed solid, raised lesion less than in 1 cm across A patch of closely grouped papules more than in 1 cm across is called a plaque
Nodule (P)	Circumscribed solid lesion with distinct edges usually more deeply rooted than a papule
Vesicle (P)	Circumscribed raised lesion less than 5 mm across filled with a clear fluid
Bulla (P)	Circumscribed raised lesion more than 5 mm across and filled with a clear fluid
Pustule (P/S)	Circumscribed raised lesion filled with purulent fluid
Macule (P/S)	Not palpable small, usually circumscribed brown, white, or red flat spot less than in 1 cm in diameter
Erosion (S)	Lesion that involves loss of the epidermis healing without scarring
Ulcer (S)	Lesion that involves loss of the upper portion of the skin (epidermis) and part of the lower portion (dermis) healing with scarring
Crust (S)	Dried collection of blood, serum, or pus overlying an impaired epidermis
Fissure (S)	Linear painful loss of skin
Lichenification (P/S)	Rough, thick epidermis with exaggerated skin lines
Atrophy (S)	Area of very thin and wrinkled skin
Scar (S)	Discolored, permanent fibrous tissue that replaces normal skin after destruction of the dermis

P primary, *S* secondary.

trisomy 21, trisomy 18, hypothyroidism, Cornelia de Lange syndrome and neonatal lupus. Differential diagnosis includes cutis marmorata telangiectatica congenita, a persistent vascular anomaly that presents at birth with marked purple reticular discoloration that may be segmental or diffuse. Associated features are skin atrophy, telangectasias, phlebectasias and ulcerations (Fig. 148.2).

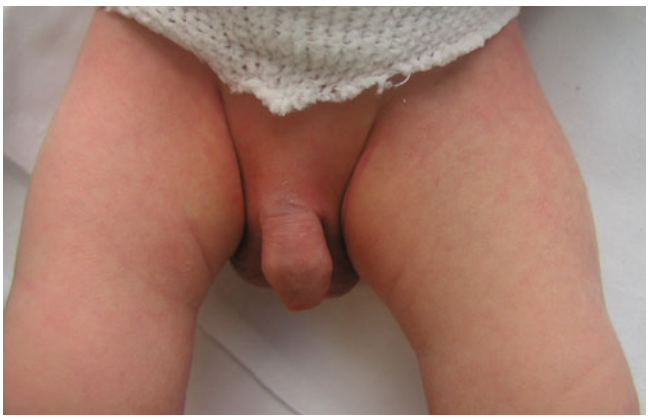

Fig. 148.2 Cutis marmorata of the lower limbs

148.3.4 Harlequin Color Change (HCC)

HCC is a harmless vascular phenomenon characterized by a transient pink to reddish discoloration that occurs in a symmetric half of an infant's body shortly after birth. Due to immaturity of the hypothalamus and inconsistent regulation of peripheral vessels, it affects 10% of newborns. It is most common in low birthweight infants. It appears as an episodic skin color change after lying on the side: a sharp line from the forehead to the pubis divides the neonate body into two vertical halves. One side turns dark red (dependent side), the other quite pale [3]. Episodes last for up to 20 minutes and disappear with movement or crying. No intervention or investigation is required.

148.3.5 Linea Nigra and Genital Hyperpigmentation (LNGH)

LNGH is a common finding in newborns, especially those with pigmented skin. The presence of linea nigra seems to be influenced by maternal hormone levels. It disappears gradually over several weeks.

148.3.6 Physiological Lanugo

Lanugo is the first body hair formed in the embryo. It is fine and soft without pigment and is usually shed in utero at 28–32 weeks of gestation. Preterm infants may show profuse lanugo on the face, limbs and trunk that is shed in the first few months of life. Congenital hypertrichosis lanuginosa (ChL) is a very rare inherited syndrome characterised by confluent, generalized overgrowth of silvery blonde to grey lanugo at birth or in early infancy. ChL continues to grow and persists throughout life. Differential diagnosis of increased neonatal hair includes several rare genetically determined disorders.

148.3.7 Transient Neonatal Conditions (Miscellaneous)

148.3.7.1 Milia

Milia are tiny keratin-filled lesions due to retention cysts of pilosebaceous follicles. Common in newborn babies, they manifest as uniform, superficial, pearly white to yellowish

domed lesions measuring 1–2 mm in diameter, usually situated around the nose and eyes and sometimes on the genitalia. Milia may also be a feature of a number of very rare genodermatoses (e.g., Bazex-Dupré-Christol syndrome). In neonatesm milia often disappear within 2–4 weeks. Persistent milia have been reported as familial disorders with autosomal dominant inheritance (Fig. 148.3).

148.3.7.2 Sebaceous Hyperplasia (SH)

SH is a common, transient, benign condition of the sebaceous glands, caused by exposure to maternal androgens in uterus. The lesions may appear as small (1–3 mm), superficial yellow-pink papules with a central pore almost always located on the tip of the nose (Fig. 148.4).

148.3.7.3 Sucking Blisters (SB)

Neonatal SB appear as unilateral or symmetrical bullae or erosions involving the upper extremities. They are self-inflicted trauma due to intrauterine sucking. The lesions are present at birth, most often on the dorsal and lateral aspects of the wrist or forearm. The infant exhibits excessive sucking. The number and the location of the lesions are diagnostic.

148.3.7.4 Subcutaneous Fat Necrosis of the Newborn (SCFN)

SCFN is an uncommon disorder characterized by firm, erythematous, painless nodules and plaques over the trunk, arms, buttocks, thighs and cheeks, manifest in full-term newborns in the first few weeks of life. SCFN is usually self-limiting, but may be complicated by hypercalcemia and other metabolic abnormalities. The pathogenesis is not known. Cold or stress-induced injury of immature fat is thought to lead to solidification and necrosis. No other organ systems are involved, unless hypercalcemia develops. Differential diagnosis is from a deep hemangioma, lipogranulomatosis, plexiform neurofibroma, and sarcoma.

148.4 Neonatal Disorders with Pustules, Blisters, Erosions and Scaly-Crusts

Vesicular and pustular disorders are common during the first months of life. Most are benign, often transient, but several serious infectious and non-infectious diseases can present in neonates. They can be diagnosed clinically because of their distinctive appearances; some cases may require simple investigations including microscopic examination of the pustule content, culture and skin biopsy.

148.4.1 Transient Sterile Pustular Disorders in Newborns

148.4.1.1 Toxic Erythema of the Newborn

This is a benign, asymptomatic skin condition occurring within 24–48 hours after birth and disappearing spontaneously after several days. It affects 30–70% of newborns. The eruption, generally starting on the trunk, is characterized by small erythematous papules, vesicles and occasionally pustules, usually surrounded by a distinctive, diffuse, blotchy, erythematous halo (Fig. 148.5). Individual lesions disappear within hours and then appear elsewhere on the body. Wright-Giemsa (WG) staining of the pustular smear reveals eosinophils. The etiology is unknown [4].

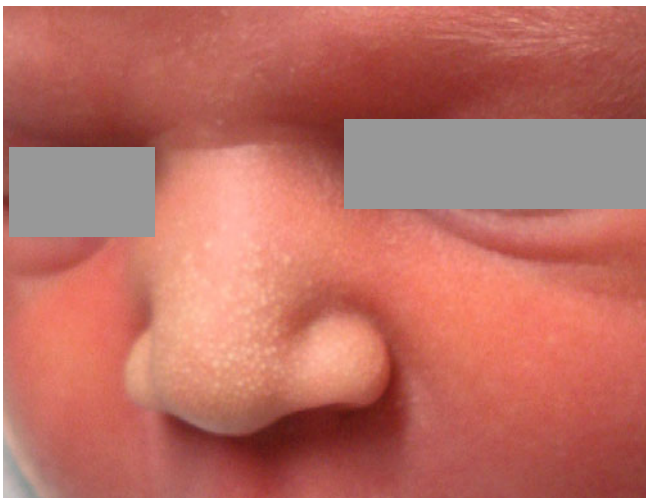

Fig. 148.4 Sebaceous hyperplasia of the nose

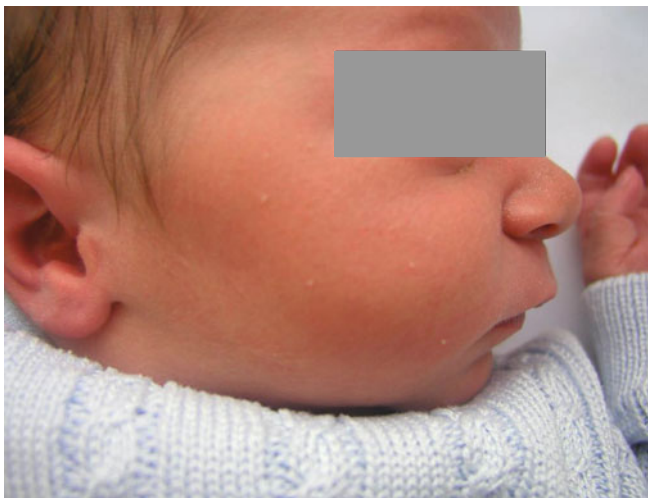

Fig. 148.3 Milia on the cheek of a newborn

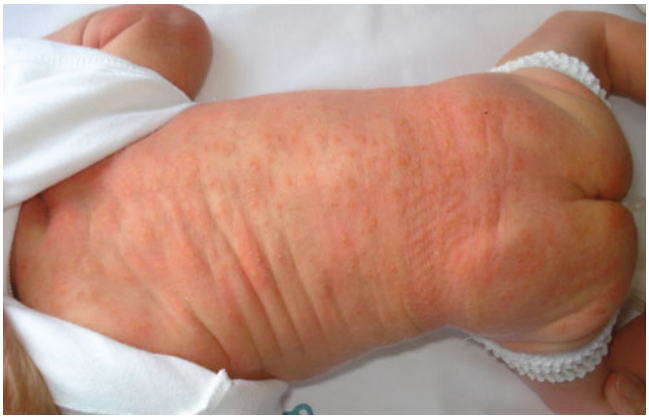

Fig. 148.5 Erythema patches and small pustular lesions of the trunk in a 3 days old newborn affected by toxic erythema

148.4.1.2 Transient Neonatal Pustular Melanosis (TNPM)

TNPM is a benign, asymptomatic, self-limiting skin condition with distinctive features characterized by vesicles, superficial pustules and pigmented macules. The lesions are generally present at birth on the chin, neck, forehead, chest and back especially in black neonates. The palms and soles are seldom involved. No systemic signs are associated with the skin eruptions. The vesicles and pustules clear up within 48 hours, while the brown macules persist for several months. Etiology is unknown. WG staining of a smear reveals many neutrophils without evidence of changes due to bacteria, yeast or viruses.

148.4.1.3 Infantile Acropustulosis (IA)

IA is a rare benign idiopathic disorder characterized by recurrent crops of 1–2 mm intensely itchy vesiculo-pustules found on the distal extremities of newborns or early infants. It is more common in black infants. Permanent recovery occurs around 2–3 years. WG staining of a smear reveals polymorphonuclear and eosinophilic leucocytes. The duration of recurrent crops is shortened by topical steroids.

148.4.1.4 Eosinophilic Pustulosis

This relatively rare disorder is characterised by recurrent crops of itchy follicular papulo-pustules located primarily on the scalp but also on the face, trunk and extremities. The pustules may be present at birth or may occur during the first few months of life and show peripheral extension with crusts and central clearing. The disorder may last days to weeks or relapse over years. WG staining of a pustular smear shows polymorphonuclear and eosinophilic leucocytes without evidence of changes due to bacteria, yeast or viruses. There may be a peripheral leukocytosis and eosinophilia. Most cases respond to topical corticosteroids.

148.4.1.5 Miliaria (M)

M is a disorder of the eccrine sweat glands that often occurs under hot humid conditions. Although it affects people of all ages, it is especially common in children and infants due to underdevelopment of their sweat glands. M is caused by blockage of the sweat glands and can be classified according to the level at which obstruction occurs: M cristallina, M rubra and M profunda. The most common form in newborns is M rubra provoking a local inflammatory reaction and the typical redness and blister-like lesions. When pustules develop in lesions of M rubra, the term miliaria pustulosa is used. M usually affects neonates aged 1–3 weeks. Superficial clear vesicles of M cristallina occurs in often confluent crops, without any surrounding erythema. Lesions tend to occur on the head, neck and upper trunk. The disorder clears up spontaneously in few days with superficial branny desquamation.

148.4.1.6 Neonatal Cephalic Pustulosis (NCP) and Neonatal Acne (NA)

NA is a true comedonal and papulopustular acne induced by maternal hormones that cause excessive facial sebum production in infants. NCP is a newer entity, consisting of a pustular condition of the head (face and/or scalp) without comedones, often occurring during the third week of life, thought to be caused by overgrowth of various *Malassezia*. NCP usually clears up without treatment in a few weeks [5].

148.5 Infective Diseases Characterized by Pustules, Blisters, Erosions and Crusts

148.5.1 Varicella (Chickenpox)

Infections due to Varicella-zoster virus (VZV) are rare before 6 months of age. Varicella of newborns is serious and potentially fatal, manifesting with disseminated red spots developing into papules and then rapidly into vesicles and pustules that finally form a scab after a few days. Congenital varicella can present with erosive lesions, but often typical depressed, pigmented or hypopigmented, cicatrized lesions are evident (Table 148.4). When varicella occurs from 8 days before delivery to 2 days after delivery, the baby may develop diffuse skin eruptions with necrotic ulcerated or hemorrhagic lesions [6].

148.5.2 Herpes Simplex

Neonatal Herpes simplex virus (HSV) infection occurs in 0.1–0.5% of live births. HSV-2 is responsible for about 70%

Table 148.4 Clinical manifestations of congenital varicella syndrome

Skin (76%)
- Dermatomal scarring
- Cutaneous defects
- Hypopigmentation

Nervous system (60%)
- Intrauterine encephalitis
- Cortical atrophy/porencephaly
- Seizures
- Mental retardation
- Autonomic instability

Eyes (51%)
- Chorioretinitis
- Cataracts
- Microphthalmia
- Horner syndrome
- Nystagmus
- Anisocoria

Musculoskeletal (49%)
- Limb/muscle hypoplasia

Other
- Intrauterine growth restriction
- Developmental delay
- Cardiovascular defects
- Gastrointestinal reflux
- Hydroureter
- Hydronephrosis
- Neurogenic bladder
- Early death

of cases and the rest are caused by HSV-1. HSV-2 infections generally result from contact with maternal genital secretions during delivery and are mostly due to primary genital infection acquired by the mother in late pregnancy. Transplacental transmission of the virus has been documented less often and may result in miscarriage or premature birth of a severely ill baby [7].

The symptoms in neonates have been classified into three forms. The classification has predictive value for outcome.

1. Skin-eye-mouth (SEM) manifestations without visceral or CNS involvement
2. Disseminated form (DISS) with sepsis-like symptoms, DIC and visceral involvement with or without CNS symptoms
3. CNS form without involvement of other visceral organs.

A fourth form, asymptomatic infection, can be added. The various forms are summarized in Table 148.5.

Skin lesions manifest at birth or within 24 hours in the case of infection in utero, and appear at about 7–14 days in babies who contracted the infection during delivery. Initial lesions are usually manifest on the head. Sometimes they become pustular but soon transform into erosions, which may be deep and hemorrhagic. On the palm or sole, vesicles or pustules tend to remain integral. Prognosis of disseminated forms is poor. Neurological sequelae are also frequent.

Differential diagnosis is seldom necessary (Table 148.6). The diagnosis can be confirmed by cytodiagnosis, immunofluorescence or viral culture. The treatment of choice is high-dose, prolonged acyclovir therapy.

148.5.3 Staphylococcal Scalded-Skin Syndrome (SSSS)

Staphylococcus aureus can cause a spectrum of exfoliative skin conditions ranging from localized bullous impetigo to generalised cutaneous involvement with systemic illness. SSSS generally affects neonates, infants and children under 5 years of age [8]. Skin manifestations appear several days after a local and often unapparent staphylococcus infection. Macular erythema first appears in periorificial patches, spreading to the whole skin surface in 12–24 h. The body becomes covered in large flaccid blisters that are easily ruptured. There is widespread epidermal lifting revealing large areas of red, shiny, exudative skin, resembling burns. The mucous membranes are spared. The child has edema, fever, weakness and altered general state and may die if antibiotic therapy (penicillinase-resistant semisynthetic penicillin) and fluid and electrolyte replacement are not begun immediately. When patients respond to treatment, recovery is achieved in 1–2 weeks without scarring. Pathogenesis is linked to entry of epidermolytic

Table 148.5 Herpes simplex virus infection in neonates: clinical presentation, mortality and morbidity. Modified from [8] and [9]

Neonatal disease	Cutaneous lesions	Visceral involvement	Vesicles at onset	Age of onset	Mortality	Disability
Skin-eye-mouth disease	Vesicles in skin, mouth or conjunctive, cornea	None	All cases	5–11 days	0%	0%
Disseminated disease	Vesicles	Sepsis-like jaundice, clotting disorders, pneumonia, hepatomegaly, DIC (meningoencephalitis)	20%	5–11 days	29%	13%
CNS disease	Vesicles	Seizures, Lethargy, Fever	50%	2–4 weeks	4%	70%
Congenital	Vesicles, scarring of skin	Brain damage, microcephaly, low birthweight	Vesicles or scars	At delivery	?	100%
Asymptomatic infection	–	–	–	–	–	–

Table 148.6 Differential diagnosis of herpes simplex virus infections in newborns

– Staphylococcal scalded skin syndrome
– Toxic epidermal necrolysis
– Aplasia cutis congenita
– Bullous epidermolysis
– Pemphigus
– Incontinentia pigmenti
– Neonatal Behçet disease
– Neonatal lupus
– Erythema annulare centrifugum
– Infantile annular erythema
– Mechanobullous disease

toxins (ET-A and ET-B) of *S. aureus* into the bloodstream [9]. Diagnosis is generally helped by the typical appearance of the syndrome, but in newborns it is necessary to distinguish scaly and eczematous erythrodermal syndromes.

148.5.4 Cutaneous Fungal Infection

Cutaneous fungal infections are quite common in newborns. The more premature the newborn, the greater the risk [10]. Clinical manifestations can be the result of infection with *Candida* species or of skin colonization with *Malassezia* species; cutaneous infection with other fungal pathogens is rare (Table 148.7). Immunocompromised and premature neonates, however, are susceptible to infection with opportunistic pathogens such as *Mucor* and *Aspergillus* species and are at higher risk of invasive infection by common pathogens such as *Candida*.

148.5.4.1 *Candida* spp

Candida albicans accounts for the majority of Candida infections in neonates.

Congenital candidiasis is acquired in utero from previous candida chorioamnionitis and generally presents at birth or in the first 2 days of life with a variety of clinical features, ranging from monilial diaper dermatitis, diffuse eruption of pustules and papulovesicles on an erythematous and scaling base with widespread erosions, to a burn-like dermatitis followed by peeling. It is generally not accompanied by thrush. The palms and soles may be involved, and onychomycosis and paronychia have been reported. In healthy full-term infants, congenital candidiasis is a benign condition that can be treated with topical antifungal agents or no therapy at all. Premature infants may present with systemic infections and widespread dermatitis. In such cases, systemic therapy is required (Amphotericin B, fluconazole or itraconazole) [11].

Neonatal candidiasis is acquired during delivery or later from nursery contacts. It usually appears after the fifth day of life and commonly manifests as oral thrush or diaper dermatitis. The typical presentation is erythematous patches with satellite papules and pustules, and scaling in intertriginous areas. Invasive fungal dermatitis presents predominantly in very low birth-weight infants as diffuse erosive, crusted and sometimes necrotic dermatitis. This condition is often fatal [12].

Table 148.7 Clinical presentation of uncommon fungal infections in neonates

Dermatophytes (*Tricophyton rubrum*, *T. tonsurans*, *T. mentagrophytes*, *Microsporum canis*, *Epidermophyton floccosum*)	Tinea corporis (scaly erythematous annular patches, with papulopustular component), tinea facei, tinea capitis (scaling erythematous scalp eruption with or without alopecia), tinea diaper dermatitis
Malassezia species (*M. furfur*, *M. sympodialis*)	Neonatal cephalic pustulosis (self limiting eruption characterized by scattered erythematous papules and pustules on the face, scalp and neck, without comedos or follicular localization) or invasive fungal disease (septicaemia and pneumonia)
Aspergillus species	Primary cutaneous infection (erythematous papules or plaques, evolving to necrotic skin lesions) or systemic infection (often fatal, may involve gastrointestinal, pulmonary and central nervous system), especially in VLBW and immuno compromised neonates
Zygomycetes (*Rhizopus*, *Mucor*, *Rhizomucor*, *Absidia*)	Primary cutaneous infection (erythematous papules, nodules or plaques, progressing to necrosis and crusted eschars) or systemic infection (often fatal, may involve gastrointestinal, pulmonary and central nervous system), especially in VLBW and immunocompromised neonates

148.5.5 Scabies

The pathognomonic skin lesion of scabies is the burrow, a white-greyish linear or tortuous ridge, with a small vesicle at the end. Several non-pathognomonic lesions can also be observed: 1–2 mm inflammatory papules, vesicles, pustules, crusts and, if the patient is not treated, red-brown nodules. These last lesions may persist for several weeks after successful therapy and represent a hypersensitivity reaction to the mite and its products. Neonatal scabies, is often characterized by a more or less diffuse and polymorphic eruption of inflammatory papules, vesicles, pustules, crusts and nodules also involving areas normally spared in adults and older children, such as the face, scalp, palms and soles [13]. Pruritus is usually absent. The nails may be involved. A frequent complication is impetiginization of lesions. The demonstration of *Sarcoptes*

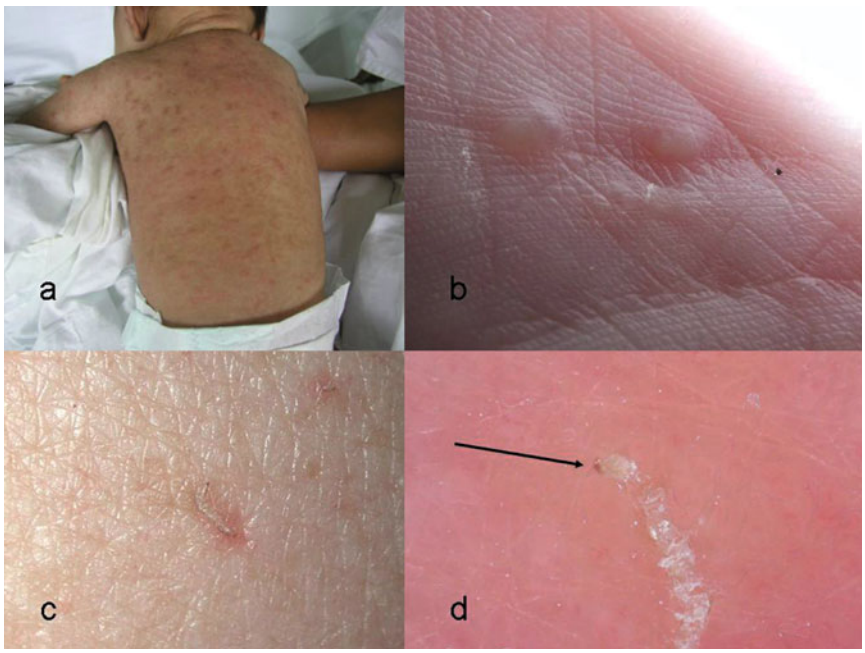

Fig. 148.6 **a** Neonatal scabies diffuse to the trunk. **b** Vesicles and scales of the palm. **c** A detail of a burrow. **d** Dermoscopic features of burrow, note the mite at the end of the burrow (*arrow)*

scabiei mites, eggs, or fecal pellets is diagnostic. A superficial scraping followed by microscopic observation of the skin samples after application of potassium hydroxide preparation seems to be the most sensible method. Dermoscopy, a new non-invasive technique is very useful in newborns, because it allows, if well performed, rapid identification of the burrows and the mite at the end of it (Fig. 148.6).

Permetrin 5% cream is regarded as a safe drug which is recommended for scabies therapy of newborn and preterm infants [14]. The parents must be treated too.

Table 148.8 Differential diagnosis of impetigo in newborns

- Neonatal syphilis
- Bullous epidermolysis
- Incontinentia pigmenti
- Neonatal pustulosis (congenital candidiasis, toxic erythema, transient pustular melanosis)
- Infantile acropustulosis
- Profuse scabies of unweaned babies
- Sweat retention syndromes

148.5.6 Bullous Impetigo

This is the most superficial form of pyoderma. In newborns and nursing babies *Staphylococcus* infection is common, manifesting with vesicobullous lesions containing serum, germs and polymorphonuclear neutrophils, which soon become pustules that erode and form yellowish brown crusts. Lesions generally start from the periumbilical regions, spreading to the trunk, scalp and exposed skin due to frequent self-inoculation from the hands. Diagnosis is generally straightforward, though in newborns various diagnoses must be considered (Table 148.8).

148.5.7 Congenital Syphilis (CS)

Syphilis is a sexually transmitted infection, caused by the bacterium *Treponema pallidum*. Vertical transmission is usually transplacental and can occur at any time during pregnancy and at any stage of disease. It is particularly likely during the first two years of the mother's infection. Pregnancies complicated by syphilis may result in intrauterine growth restriction, non-immune hydrops fetalis, stillbirth, preterm delivery, and CS.

CS occurs in over 60% of infants born to untreated infected mothers. The timing of infection in utero impacts whether early signs are present at birth. For live-born infants, CS is divided into early or late disease depending on whether it presents before or after 2 years of age. Two-thirds of infants with CS are asymptomatic at birth, but signs appear within 3–8 weeks after birth. Neonates born with overt manifestations of infection at delivery are usually severely affected and with a poor prognosis. Lesions are usually inflammatory and exudative. Early manifestations of CS involving multiple organ systems are described in Tables 148.9 and 148.10. Neonates show irritability, failure to gain weight or failure to thrive and nonspecific fever. The most striking lesions affect the mucocutaneous tissues and bones [15]. Dermatological findings are quite variable, although palmar/plantar, perioral, and anogenital regions are classically involved. Mucous

Table 148.9 Clinical manifestations in early congenital syphilis (presentation before 2 years of age)

Manifestations	Incidence %
Prematurity	Depends on whether vertical transmission occurred
Jaundice	15
Hepatosplenomegaly	33–100
Generalized lymphadenopathy	20–50
Skeletal involvement (osteochondritis, epiphysitis and periostitis)	70
Neurological symptoms and signs (hydrocephalous, cranial nerve palsies)	40–60
Meningitis	10
Muco-cutaneous lesions	30–60

Table 148.10 Cutaneous lesions in early congenital syphilis (presentation before 2 years of age)

Lesions	Distribution
Ragadized mucous patches	Mouth and perianal area
Snuffles	Nose
Condilomata lata	Intertriginous and periorificial areas
Maculopapular desquamative rash	First palms and soles; then extremities, face, diaper area and trunk
Vesicular-bullous rash	Palms and soles

patches, rhinitis, and warty lesions are highly characteristic features of tegumental involvement in early CS. Smooth, round, moist mucous patches involve the mouth and perianal area. Rhinitis with profuse and occasionally bloody rhinorrhea, is invariably present in symptomatic babies. The infant may have a characteristic "old man" look with fissured lesions around the mouth. Moist, flat warty lesions (condilomata lata) may develop in intertriginous and periorificial areas. Mucocutaneous lesions usually appear between 2 and 6 weeks of age. Snuffles are followed quickly by a diffuse maculo-papular desquamative rash that involves extensive sloughing of the epithelium and begins on the palms and soles and spreads over the extremities, face, diaper area and trunk. In contrast to acquired syphilis, a vesicular rash and bullae may develop. All these lesions are highly infective. The rash appears over seven days and may remain for several months as copper-colored lesions. Screening of neonates at risk of syphilis include specific treponemal and non-treponemal studies. Serological tests that detect IgG may be positive due to passive transfer of maternal antibodies. A positive anti-treponemal EIA IgM is indicative of congenital infection. Diagnosis of CS is made when the mother of an infant has a reactive treponemal and non-treponemal serologic test with characteristic clinical findings in the infant or when *T. pallidum* is demonstrated by dark-field microscopy or immunoflorescence from placental, skin or umbilical lesions. Infants with clinical signs of illness or suggestive serologic test results also should have lumbar puncture with CSF specific analysis and long-bone X-rays. Serological tests may be negative in infants infected in late pregnancy and should be repeated. Early diagnosis and treatment with high-dose penicillin prevents late complications of syphilis.

148.6 Non-Infective Blistering and Pustular Conditions

148.6.1 Inherited Blistering Diseases

148.6.1.1 Epidermolysis Bullosa (EB)

This is a group of rare inherited disorders characterized by blister formation caused by minor frictional trauma [16]. These diseases are the result of various genetic defects in essential dermal-epidermal adhesion molecules, which anchor basal keratinocytes to the underlying papillary dermis. Thirty subtypes of EB are described and have been divided into four major forms: simplex (EBS), junctional (JEB), dystrophic (DEB) and Kindler syndrome, based on clinical findings, pattern of inheritance, presence or absence of scarring and particularly the level within which blisters arise (Table 148.11) [17, 18].

In all twelve subtypes of EBS, blistering, often congenital from passage through the birth canal, is superficial and heals without scarring. The most common subtypes are inherited in an autosomal dominant pattern. In newborns, blistering and

Table 148.11 Major EB types and subtypes

Histopathology	Major EB type	Major EB subtypes	Targeted protein(s)
Intraepidermal cleavage	EBS	Suprabasal EBS	Placophilin-1; desmoplakin; others
		Basal EBS	Keratins 5,14; plectin; α6β4 integrin
Intra-lamina lucida cleavage	JEB	Herlitz-JEB	Laminin-332 (laminin-5)
		JEB, other	Laminin-332; type XVII collagen; α6β4 integrin
Sub-lamina densa cleavage	DEB	Dominant DEB	Type VII collagen
		Recessive DEB	Type VII collagen
Mixed	Kindler syndrome	–	Kindlin-1

EB epidermolysis bullosa, *EBS* epidermolysis bullosa simplex, *JEB* junctional epidermolysis bullosa, *DEB* dystrophic epidermolysis bullosa.

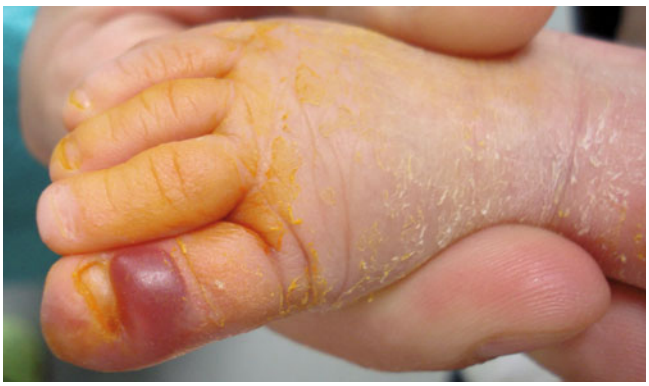

Fig.148.7 A blister of the big toe in a newborn affected by epidermolysis bullosa. Orange colour is due to topical antiseptics

large erosions primarily affect the feet, hands, neck and lower legs (Fig. 148.7). The severity of phenotypes varies greatly.

Junctional EB is an autosomal recessive disorder with seven subtypes, the most common of which is called Herlitz-JEB. This very severe variant of EB is associated with 50–80% mortality in the first two years of life. These infants have massive erosions that heal very slowly, often forming thick granulation tissue. The nail plates are often absent at birth, replaced by abundant granulation tissue. These infants are prone to infection, electrolyte imbalance, temperature instability, and high metabolic demand. Airway disease, growth retardation, eye problems and gastrointestinal diseases are often associated [19].

Dystrophic EB is an inherited autosomal dominant and recessive. In the latter form, much more severe, widespread blistering begins at birth and results in contractures with severe scarring and milia formation due to split trough pilosebaceous or sweat ducts. Fusion of digits and autoamputations lead to classic "mitten deformities". Systemic involvement may be severe, with gastrointestinal complications being the most common. Nail changes are invariable in all forms of DEB.

Diagnosis may be established by electron microscopic examination of a new blister, immunofluorescence mapping and mutational analysis. Management of all forms of EB consists of good wound care with antibacterial creams and non-adherent dressings, prevention of trauma and prevention and treatment of infection. In severe forms, newborns should initially be nursed naked in a humified crib on non-adherent material and with barrier nursing. Nasogastric tubes, tourniquets, adhesive urine collection bags, identification bands and pacifiers should all be avoided. Some evidence of phenotypic reversion of JEB keratinocytes by somatic gene therapy has been reported recently.

148.6.1.2 Mastocytosis

The term mastocytosis denotes a heterogeneous group of disorders characterized by abnormal growth and accumulation of mast cells (MC) in one or more organ systems [20]. The diagnosis of cutaneous mastocytosis (CM) is based on typical clinical and histological skin lesions and absence of definitive criteria of systemic involvement. Most patients with CM are children and have maculopapular cutaneous mastocytosis/urticaria pigmentosa (UP) [21]. In UP, rarely present at birth, the skin shows a maculopapular rash, often with pigmented lesions, wheal and flare phenomenon with stroking and a positive "Darier's sign" (Fig. 148.8 and Tables 148.12, 148.13)

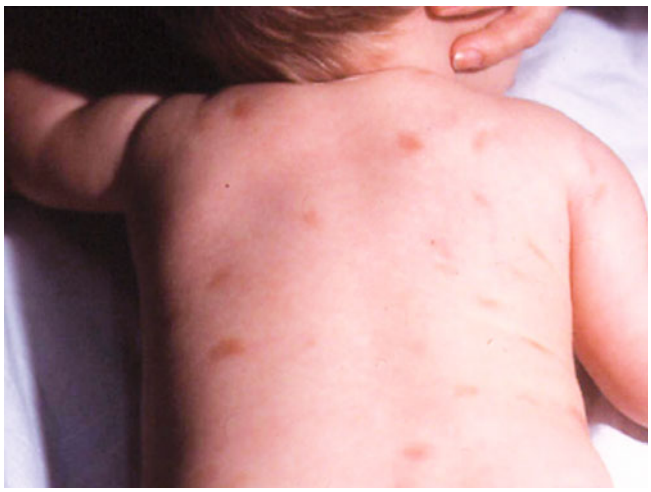

Fig. 148.8 Diffuse cutaneous mastocytosis

Table 148.12 Classification of cutaneous mastocytosis

Variants	Criteria	MC burden	Grade	Typical findings
Urticaria pigmentosa (UP) Maculopapular cutaneous mastocytosis (MPCM)	Maculopapular lesions	Low	Low	–
Subvariants:				
Typical UP	Maculopapular	Low	Low	Vast majority of cases
Plaque-form	Plaques	Low	Low	Often found in infants
Nodular	Mastocytoma-like lesions (≥2)	Low (rarely high)	Low	Rare
Telangiectasia macularis eruptiva perstans (TEMP)	Telangectasias, macules	Low	Low	Rare
Diffuse cutaneous mastocytosis (DCM)	Erythrodermic rash	Low or high	Low	More diffuse patterns
Mastocytoma of skin	Unifocal (local) tumor	Low	Low	–

Table 148.13 Proposed criteria to diagnose mastocytosis

Cutaneous mastocytosis	Typical skin lesions, typical clinical signs (UP/MPCM, DCM, mastocytoma) and positive histology with typical infiltrates of MC (diagnostic infiltrate-pattern: multi/focal or diffuse)
Systemic mast cell disease "SM criteria"	Diagnosis may be rendered if one major plus one minor or three minor criteria are fullfilled
Major criterion	Multifocal dense infiltrates of MC (>15 MC aggregating) detected in sections of bone marrow and/or of other extracutaneous organ(s) by tryptase-immunochemistry or other special stains
Minor citeria	a. In biopsy sections (of bm or other extracutaneous organs), more than 25% of the MC in the infiltrate have atypical morphology or more than 25% of MC in the aspirate smear are immature or atypical b. Mast cells co-express CD117 with CD2 and/or CD25 c. Detection of a c-kit point mutation at codon 816 in bone marrow or blood or other extracutaneous organ(s) d. Serum total tryptase persistently >20 ng/mL (not valid in case of an associated hematologic clonal non-MC lineage disease, AHNMD)

MC mast cells, *UP* urticaria pigmentosa, *MPCM* maculopapular cutaneous mastocytosis, *DCM* diffuse cutaneous mastocytoses.

[22]. Other less frequent forms of CM, diffuse cutaneous mastocytosis (DCM) and mastocytoma of the skin, are usually congenital [23, 24]. Mastocytomas appear as one or several, isolated, skin-coloured to light yellow-brown, 1–5 cm macules, nodules, or plaques, typically on the trunk or extremities (Fig. 148.9). Spontaneous or friction-induced blistering is common and bullous lesions may also develop. In diffuse cutaneous mastocytosis, a more diffuse erythtrodermic rash is found and some patients may show generalized thickening of the skin, often described as "doughy", or additional nodular lesions. Sometimes, rubbing, bullous lesions may develop. All forms of cutaneous mastocytosis improve with time, sometimes healing completely at puberty. The major mediators secreted by mast cells are histamine, heparin and prostaglandins, the effects of which account for many of the clinical features of mastocytosis. The predominant symptom in all is pruritus. Additional mediator-related symptoms include flushing, hypotension, fatigue, diarrhea, vomiting and abdominal pain.

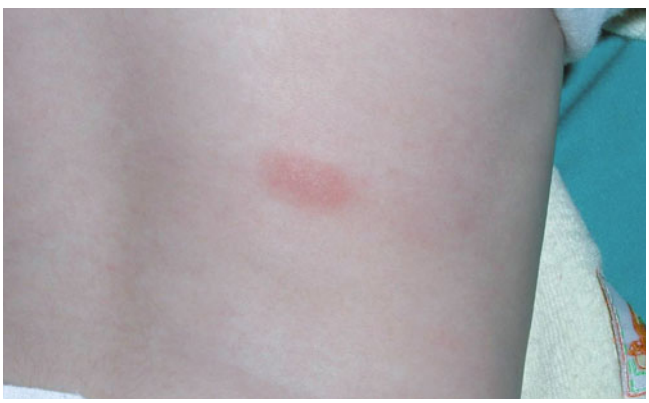

Fig. 148.9 Solitary mastocytoma of the trunk

148.6.1.3 Incontinentia Pigmenti (IP)

IP is a rare, X-linked dominant genodermatosis, usually lethal in males. It affects the skin and its appendages, bones, eyes and central nervous system [25, 26]. Cutaneous lesions usually appear at birth or in the first days of life and have three distinct morphological stages (vesiculopustular, verrucous and pigmented) with stage one occurring in the neonatal period [27]. Initially, there are clear-to-yellow vesicles, measuring 2–4 mm, clustered in a linear configuration, mostly on the trunk and distal limbs. They may sometimes be pustular or crusted. At this stage, peripheral blood eosinophilia is often associated. Vesicular and pustular lesions are replaced within several weeks by grey to brown, linear and swirled verrucous papules and plaques, the second stage of the disease, and after years by brown to grey macular hyperpigmentation in streaks and whorls.

The diagnosis of IP is based on clinical findings [28]. It should be considered when inflammatory linear groups of vesicles are seen in a newborn female infant. Biopsy of a blister reveals a subcorneal vesicle filled with eosinophils. Lyonisation accounts for the linear pattern of this disease, typical of mosaic disorders [29]. Ocular, skin appendage, dental and skeletal abnormalities may also occur [30]. No specific treatment is required for the skin lesions, as spontaneous healing usually occurs.

148.6.1.4 Neonatal Pemphigus Vulgaris (NPV)

The term pemphigus refers to a group of diseases characterized by cutaneous or mucosal blisters and erosions, and antiepidermal autoantibodies against desmoglein 1 and desmoglein 3 [31, 32]. NPV is caused by passive transfer of maternal IgG antibodies across the placenta in pregnancy from mothers with PV or pemphigus foliaceus [33]. Lesions are usually present at birth, ranging from flaccid bullae and/or vesicles to large erosions on the whole body. Mucous membranes are rarely involved. Histopathological findings include intraepithelial blisters and acantholytic cells. Direct immunofluorescence is always positive, and indirect immunofluorescence almost always positive. Neonatal prognosis is excellent: non-corticosteroid ointments usually produce prompt epithelization of the erosive lesions and skin eruptions generally disappear within 3 weeks.

148.6.1.5 Herpes Gestationis (HG)

HG is an uncommon, autoimmune, bullous disease that appears during the second or third trimester of pregnancy [34]. It almost invariably recurs in subsequent pregnancies. Anti-180 kDa bullous pemphigoid autoantibodies are the cause. The disorder may be evident at birth or within several hours in neonates from mothers affected by HG. The skin eruption consists of red macules or papules, often progressing to vesicles or bullae. The typical rash usually starts around the umbilicus and spreads over the trunk and extremities. Rapid improvement occurs over few days with spontaneous recovery by one month of age.

148.7 Disease with Systemic Involement Presenting with Pustulas, Blistering and Erosions

148.7.1 Acrodermatitis Enteropathica (AE)

This rare autosomal recessive disease is caused by the impaired absorption of zinc in the gastrointestinal tract. Signs and symptoms of AE may be neurological-behavioral, gastrointestinal (diarrhea), ophthalmologic, infectious or dermatological. Cutaneous manifestations of zinc deficiency are obligatory and diagnostic. Skin lesions are erythematous with peripheral crusting. Margins are well defined, progressing to vesicobullous, erosive or pustular lesions. The typical distribution is periorificial and acral (Fig. 148.10). AE patients need zinc supplementation for life.

The main differential diagnoses are diaper dermatitis, chronic mucocutaneous candidiasis, atopic dermatitis, impetigo, epidermolysis bullosa, psoriasis and seborrhoeic dermatitis.

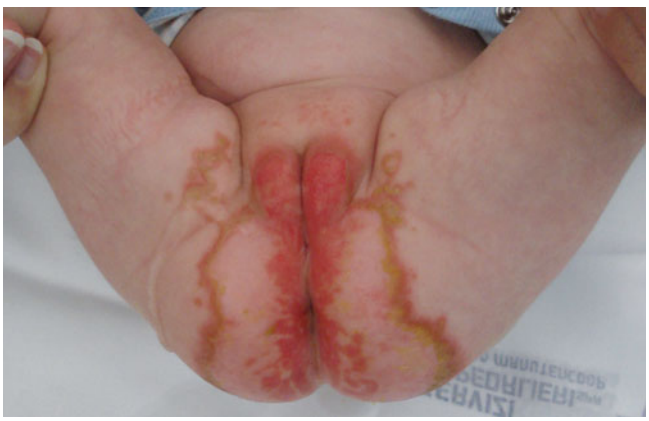

Fig. 148.10 Acrodermatitis enterophatica with perianal and genital lesions

Table 148.14 Clinical characteristics of hyper IgE syndrome

- Pustular and eczematoid rash
- Blisters
- "Cold" abscesses
- Pyogenic pneumonia
- Pneumatocoele
- Bronchiectasis
- Eczema
- Mucocutaneous candidiasis, disseminated *Cryptococcus* and *Histoplasma* infection
- Peak serum IgE > 2000 IU/mL
- Eosinophilia
- Increased incidence of lymphoma (both Hodgkin and non Hodgkin)
- Scoliosis, osteopenia, minimal trauma fractures, hyperextensibility
- Cranyosynostosis
- Arterial aneurisms
- Facial asymmetry, deep-set eyes, prominent forehead
- Retained primary dentition
- High arched palate
- Alteration of oral mucosa and tongue

148.7.2 Hyper-Immunoglobulin-E Syndrome (HIES)

Hyper-immunoglobulin-E syndrome, also called Job's syndrome, is a very rare primary immunodeficiencies , characterized by chronic eczematous dermatitis, recurrent skin and respiratory tract infections, markedly elevated serum IgE (>2000 IU/mL), and a variety of connective tissue and skeletal abnormalities (Table 148.14) [35]. In almost all cases, an eczematoid rash, first affecting the face, scalp and upper trunk and later progressing over the whole body, with papules or pustules, usually due to *S. aureus* infection, manifests in the first month of life. Blistering is a classic finding in this disease. To control the rash antibiotic prophylaxis is indicated.

148.7.3 Langerhans Cell Histiocytosis (LCH)

LCH is a rare group of disorders of the monophagocytic system, with a peak incidence at 1–4 years of age, characterized by proliferation of a distinct cell type that is CD1a, S100, CD207 positive and contains Birbeck granules in various organs. Cutaneous involvement is encountered in 50% of the cases. It includes four main clinical forms: Letterer–Siwe disease, Hand–Schuller–Christian disease, eosinophilic granuloma and congenital self-healing Langerhans-cell histiocytosis (CSHLCH). CSHLCH is the most frequent found in newborns. It presents at birth or in the neonatal period with papulonodules or less commonly with vesiculopustules and blisters. It usually affects otherwise healthy infants. Systemic involvment must be excluded anyway [36].

148.7.4 Porphyrias

The porphyrias are a group of metabolic disorders of heme biosynthesis that may present with acute neurovisceral symptoms, skin lesions or both [37]. Of the cutaneous porphyrias, erythropoietic protoporphyria (EPP), congenital erythropoietic porphyria (CEP), hepatoerythropoietic porphyria (HEP), and the hereditary form of porphyria cutanea tarda can present in infancy.

148.7.4.1 Erythropoietic Protoporphyria EPP

EPP is characterized by acute photosensitivity, with rare subepidermal bullae in exposed areas. It may occur in neonates during phototherapy. Liver disease is an uncommon, potentially fatal, complication of EPP.

148.7.4.2 Congenital Erythropoietic Porphyria (CEP)

CEP presents soon after birth with typical reddish urine, severe photosensitivity, hypertrichosis of the face and extremities, mechanical fragility leading to formation of bullae, erythrodontia and hemolytic anemia. The blisters cause scarring, dyschromia and deformations.

148.7.4.3 Hepatoerythropoietic Porphyria (HEP)

HEP appears at birth or in the first years of life. It is characterized by early severe photosensitivity resulting in bullae, erosions, crusts in sun areas and severe hypertrichosis. Patients must be protected from light by clothing, sunscreen and beta-carotene. Whenever photosensitivity is observed in a neonate, porphyrin levels in red cells, plasma, urine and faeces should be measured.

148.7.5 Skin Manifestation in Down Syndrome

Skin manifestations are rare but characteristic. There have been few reports in the literature describing crusted, erythematous, vesiculopustular eruptions associated with trisomy 21 concentrated on the face, and spreading to the trunk and extremities.

148.8 Red Scaly Skin in the Newborn

The term neonatal or infantile erythroderma denotes diffuse erythema with variable scaliness affecting more than 90% of the body surface. A number of major conditions can present with erythroderma in the neonate or young baby (Table 148.15) [38–39]. Prognosis is poor, especially in babies with immune deficiency or chronic disease (mortality 26.2%). Severe skin diseases persist in 60% of survivors [40].

148.8.1 Immunodeficiencies (ID)

Because of the protective effect of maternal immunity, congenital ID syndromes are rarely symptomatic at birth. It is now clear that what was described in the past as Leiner's disease is a phenotype of non-congenital early-onset erythroderma, diarrhea and failure to thrive.

148.8.1.1 Omenn's Syndrome

Omenn's syndrome is an autosomal recessive form of severe combined ID characterized by exfoliative erythroderma that occurs at birth or in the early neonatal period in association with diffuse alopecia, lymphadenopathy, hepatosplenomegaly, recurrent infections and failure to thrive.

148.8.1.2 Graft Versus Host Reaction

Graft versus host reaction from maternal engraftment can occur as a result of the transplacental passage of maternal lymphocytes during intrauterine or postnatal transfusions. In immunocompetent newborns, clinical manifestations are minimal, often resulting in only a transient macular rash. In contrast, in newborns with congenital ID, GVH reaction occurs within the first two or three weeks of life with fever, eosinophilia, lymphocytosis, lymphadenopathy and hepatosplenomegaly, but may be present at birth as a morbilliform rash which evolves into erythroderma [41].

In the first weeks of life, ID should be suspected in cases in which the presenting features include pruritic erythroderma combined with skin induration, nondysplastic alopecia of hair, eyelashes, and eyebrows, widespread lymphadenopathy, and deep systemic infection. Skin infiltration is strongly indicative of Omenn's syndrome or ID, although true atopic dermatitis with marked lichenification can have a similar appearance. An association with diarrhea and extreme failure to thrive should also suggest the diagnosis. Histological findings in these cases are helpful.

148.8.2 Ichthyoses

Congenital ichthyoses are a large and heterogeneous group of monogenic disorders of cornification, sometimes associated

Table 148.15 Neonatal erythroderma: more common causes and management

Disease	Clinical features	Etiology	Treatment
Infections			
SSSS	Preceding purulent infection; skin tenderness; superficial blisters; positive Nikolsky sign	Bacterial infection	Antibiotic, contact tracing
Toxic shock syndrome	Concomitant maternal infection; skin tenderness; hypotension/shock	Bacterial infection	Antibiotic, intravenous Ig
Congenital cutaneous Candidiasis	Maternal vaginal candida infection; oral cavity spared; may have paronychia and nail dystrophy	Mycological infection	Antimycotics
Ichthyoses			
Non-bullous ichthyosiform erythroderma	Collodion baby; when shed leaves disseminated ichthyosiform scaling	Transglutaminase 1 gene defect and other genetic defects	Emollients, bland keratolytic creams
Bullous ichthyosiform erythroderma	Superficial blistering and erosions; ichthyosiform erythroderma; family history; linear epidermal nevus parents or sibling	Keratine gene 1,2,10 defects	Emollients, bland keratolytic creams
Netherton's syndrome	Diarrhea; failure to thrive; atopy; sparse hair, trichorrhexis invaginata (bamboo hair)	Genetic defects	Emollients; adequate hydration
Conradi-Hunermann syndrome	Linear and swirled patterns	Genetic defects	Emollients
Drugs			
Ceftriaxone	Infection for which it was prescribed	Allergic reaction	Reversible on discontinuation
Vancomycin	"The red man syndrome" sudden hypotension and erythema	Allergic reaction	Reversible on discontinuation
Others			
Seborrhoeic dermatitis	Cradle cap, accentuation in skin folds of neck, axilla and nappy area	Multifactorial	Moisturising agents; miconazole-hydrocortisone ointment; protective cream nappy area
Atopic dermatitis	Encrusted eczema on the scalp and face; generalized eczematous skin; family history for atopy	Allergy	Weak topical steroid; systemic antibiotics if skin infected
Psoriasis	Erythematosquamous patches; can be pustular; may have positive family history	Multifactorial	Bland emollient creams, wet dressing helpful
Pityriasis rubra pilaris	Similar to psoriasis; follicular accentuation; skin thickening of palms and soles; may have positive family history	Corneification disorder	Along same lines as psoriasis
Diffuse mastocytosis	Darier's sign often with blistering	Abnormal proliferation of mastocytes	H1 and H2 antagonists; oral sodium cromoglycate; avoidance of substances with potential for mast cell degranulation

Modified from [42].

Table 148.16 Common versus rare forms of non-syndromic ichthyosis

	X-linked ichthyosis	Lamellar ichthyosis (or CIE)	Bullous ichthyosis (or EHK)
Inheritance	XR	AR	AD
First appearance	Infancy (at birth?)	At birth	At birth
Affected gene(s)	STS	TGM1, Ichthyin, ALOXE3/12B, etc	KRT 1,2,10
Histopathology	Retention hyperkeratosis	Hyperproliferative hyperkeratosis	Hyperproliferative hyperkeratosis
Cutaneous clinical features	Brown scales also on the trunk	Collodion baby; dry, scaly skin; ectropion; hypohidrosis, erythema	Intense blistering at birth, verrucous hyperkeratosis and erythema

CIE congenital ichthyosiform erythroderma, *EHK* epidemolytic hyperkeratosis, *STS* steroid sulphatase gene, *TGM1* transglutaminase 1 gene, *ALOX* lipoxygenase gene, *KRT* keratin gene.

with systemic symptoms (Table 148.16). A common feature is rough, dry scaly skin. Some patients have complex ichthyoses, with systemic manifestations, mainly involving the CNS, immune system and skeleton.

The two most common forms of ichthyosis, autosomal dominant ichthyosis vulgaris (IVU) and X-linked recessive ichthyosis (XRI) start early in life, but are not present at birth. Lamellar ichthyosis, with its variants non-bullous icthyosiform erythroderma and bullous icthyosiform erythroderma, are invariably present at birth with variable degrees of erythroderma [43].

148.8.2.1 Non-Bullous Ichthyosiform Erythroderma

Non-bullous icthyosiform erythroderma, an autosomal recessive congenital ichthyosis, is characterized by fine white-greyish scales and erythroderma. Many patients suffer from deep skin fissures, and some develop flexion contractures. About 90% of patients with non-bullous ichthyosiform erythroderma present as "collodion babies" enveloped in a glistening membrane resembling sausage skin that may produce ectropion, lip eversion, nasal obstruction, temperature instability and fluid loss. The membrane peels off in days or weeks and this may be a presentation of various forms of ichthyosis besides congenital icthyosiform erythroderma. The most severe phenotype of autosomal recessive congenital ichthyosis is "harlequin ichthyosis", in which neonates are covered with plate-like scales and massive hyperkeratosis, leading to severe disfigurement, particularly of the face. The skin soon splits forming bleeding fissures, and then peels-off leaving red scaly skin.

148.8.2.2 Bullous Ichthyosiform Erythroderma

Bullous ichthyosiform erythroderma, presents with generalised erythema and superficial blisters. Bullous lesions become less prominent as the infant grows, and may eventually disappear completely. These children later develop typical ichthyosiform hyperkeratosis, with hyperkeratosis more pronounced in the flexural areas, in which the dark, warty scales often acquire a ridged pattern with frequent maceration that causes a strong unpleasant odor.

148.8.2.3 Netherton's Syndrome

Netherton's syndrome is a complex ichthyosis with high mortality (30–40%) characterized by a triad of generalized exfoliative dermatitis, sparse hair with trichorrhexis invaginata (bamboo hair), and atopic features. It usually presents at birth as erythroderma. Patients are atopic and suffer from recurrent angio-edema and urticaria.

148.8.3 Drugs

Although many drugs can determine an erythematous maculo-papular rash, erythroderma has only been described in neonates treated with ceftriaxone and vancomycin.

148.8.4 Seborrhoeic Dermatitis (SB)

Infantile SB is a papulosquamous eruption that typically develops during the first 12 weeks of life. The characteristic feature is yellowish, inflammatory, scaling of the scalp (cradle cap), which spreads over the face, including the forehead, eyebrows, ear, and nose (Fig. 148.11). Lesions often involve skin folds of the neck, postauricular regions, midtrunk, umbilicus, axilla, and groin. The scales may be absent in the flexures, and secondary candidiasis is common.

Other forms of presentation include a psoriasiform variant or rarely erythroderma. Pityrosporum ovale is usually found in this disorder. SD is often asymptomatic and self-limiting after the early months of life. Scales on the scalp may be treated with mild baby shampoo, and a soft-bristled toothbrush.

148.8.5 Atopic Dermatitis

Although about 18% of all children develop skin symptoms within the first 4 weeks of life, this condition rarely presents in the neonatal period with an erythrodermic rash. These patients are likely to go on to difficult long-term disease. In young infants, the primary lesion of atopic eczema is

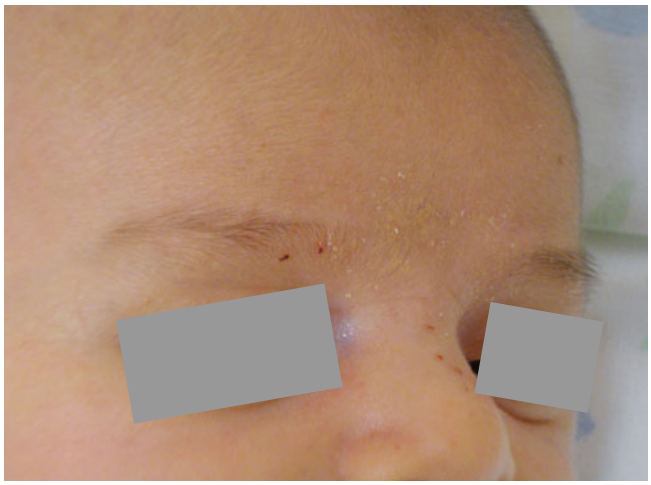

Fig. 148.11 Seborrhoeic dermatitis of the glabella in a 10 day old baby

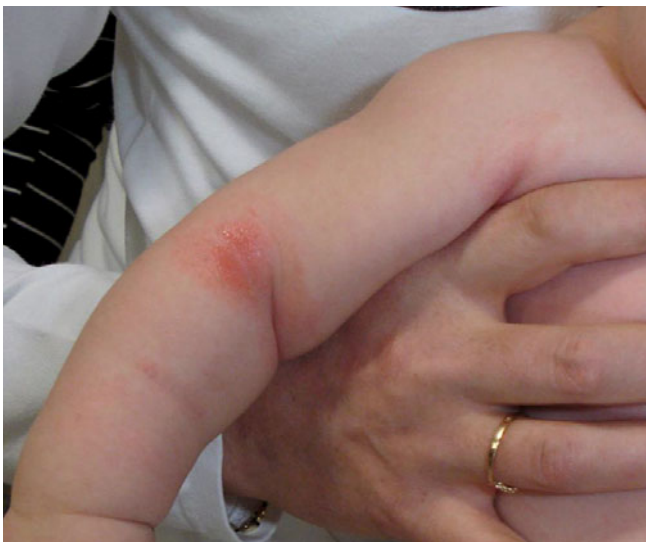

Fig. 148.12 Flexural lesion of the upper limb in a 30 day old boy affected by atopic dermatitis

frequently vesicular and exudation is common. Atopic dermatitis more often involves the face, especially the cheeks, flexural creases of the limbs, and usually spares the nappy area (Fig. 148.12). In atopic dermatitis, itching is usually not apparent until 2–3 months of age. The total IgE and eosinophil count, usually considered to be markers of atopy, are neither constant nor specific. Management involves emollients and careful use of weak topical steroids, sometimes with wet dressing.

148.8.6 Psoriasis

Psoriasis may rarely present at birth or shortly after as generalized erythroderma, sometimes evolving into pustular psoriasis. A dramatic form of psoriasis seen in babies is napkin (diaper) psoriasis with dissemination. Starting with a bright-red napkin rash, this form of the disease later develops with small scaly patches beyond the margins. An explosive psoriasic eruption develops on the scalp and face and all over the trunk, self-limiting in a few weeks.

148.8.7 Pityriasis Rubra Pilaris (PRP)

This disease is characterized by scaly erythematous plaques similar to psoriasis and follicular hyperkeratosis. They may coalesce to larger plaques and become generalized as erythroderma. Palmoplantar keratoderma is frequently associated. Congenital erythrodermic PRP is inherited as an autosomal dominant trait and, unlike the acquired forms of PRP, tends to last a lifetime.

148.9 Neonatal Lupus Erythematosus (NLE)

NLE is a rare condition caused by transplacental passage of maternal antibodies against SS-A/Ro and SS-B/La [44]. Only 1% of infants with positive maternal autoantibodies develop NLE, when the mothers have clinical or subclinical LES, LECS or Sjogren syndrome. Most infants have heart and skin manifestations, but some may also have blood and liver involvement (Table 148.17). Skin lesions occur in the first month of life or may be present at birth. Cutaneous findings usually disappear at about the sixth month of life. Lesions resemble those of subacute cutaneous lupus erythematosus (SCLE): mild scaling erythematous plaques with well-defined margins, often annular, predominately affecting the scalp, neck and face [45]. The plaques are typically periorbital, but may appear on the trunk or extremities. In male babies they are more often crusted. Solar exposure can precipitate the eruption. In some cases, erythema spreads over the entire body. Thrombocytopenia and hepatic disease may also be common. Healing tends to be associated with mild cutaneous atrophy, with or without teleangectasia. The histologic aspect of the cutaneous lesions is similar to that of SCL. NLE is the most common cause of congenital complete heart block and is associated with significant morbidity and mortality. Treatment of the mother with corticosteroids early in pregnancy may prevent heart block.

Table 148.17 Clinical manifestations of neonatal lupus erythematosus

Organ	Clinical manifestations
Skin	– Annular-polycyclic erythematous plaques – Persistent telangiectasias, atrophy or pigmentation (less frequent) – Confluent erythema around the eyes, giving an "owl eye" or "eye mask" appearance
Heart	– Congenital heart block with or without NLE Cardiac blocks usually develop in utero between weeks 18–20 of pregnancy. Once established it is irreversible. Most surviving children require permanent pacemaker implantation – Cardiomyopathy, present in some cases usually in association with heart block, is evident at or prior to birth and is often life threatening
Blood	– Thrombocytopenia – Pancytopenia – Anemia – Neutropenia – Hypocomplementemia
Liver	– Hepatomegaly with mild elevation of aminotransferase levels – Neonatal hemochromatosis – Cholestasis with conjugated hyperbilirubinemia and minimal elevation of transaminase
Others	– Macrocephaly – Hydrocephalus – Gastrointestinal bleeding

148.10 Congenital Disorders of Melanin Pigmentation

Depigmented nevi simplex type 1 (DNS type 1) is the most frequently found white spot on newborns and infants [46]. Up to 1 in 130 otherwise healthy newborns have at least one depigmented nevus present at birth, most commonly on the trunk, usually measuring 2–3 cm. The lesions are well circumscribed with irregular borders, non-progressive, lighter than the surrounding skin but seldom white. Depigmented nevi simplex type 2 (DNS type 2) are larger than 10 cm, block-like or linear lesions, with or without whorls. They may or may not follow Blaschko lines, are usually present at birth, involve one or several body segments. There is no associated pathology.

Metameric or segmental nevi have also been described. In such cases, the main differential diagnosis is metameric or segmental vitiligo, but this condition does not occur at birth. Rarely depigmented maculae are a part of multisystemic disorders (e.g., Ito's hypomelanosis or neurocutaneous melanosis).

148.10.1 Nevus Anemicus

Nevus anemicus resemble an achromic nevus, 1–3 cm in diameter, round, slightly hypochromic and with a ragged outline. If pressure is applied with a convex glass, the nevus disappears, and if stroked, no flare is elicited.

148.10.2 Neurofibromatosis

Neurofibromatosis 1 (NF1) is a common neurocutaneous condition with autosomal dominant inheritance [47]. Some cutaneous features of NF are present at birth, others are age-related. Café-au-lait spots are usually the initial clinical manifestation of NF1 and may be present at birth or appear in infancy. They may be found anywhere on the skin but rarely on the face and scalp. They tend to increase in number and size throughout early childhood. Plexiform neurofibromas, found in at least 25% of patients with NF1 are also congenital. They feel like a bag of worms and cause gross disfigurement. They may also affect deep structures and cause pulmonary or vascular involvement. Early in life, the lesions may only be recognized as soft tissue enlargement or a patch of cutaneous hyperpigmentation with or without hypertrichosis. The other typical cutaneous findings of NF appear later.

148.10.3 Tuberous Sclerosis (TS)

The TS complex is an autosomal dominant multisystem neurocutaneous disorder characterised by widespread hamartomas in several organs, including the brain, heart, skin, eyes, kidney, lung and liver [48]. Most features of tuberous sclerosis only become evident after 3 years of age. The earliest cutaneous lesion to appear in TS is the hypopigmented macule, also called an "ash leaf" macule, frequently present at birth. These lesions often come in other shapes: they may be round, but most are polygonal, usually 0.5–2.0 cm in diameter, resembling a thumbprint. Another manifestation of TS that may be seen at birth is a hypopigmented tuft of hair. However, these findings are not pathognomonic of TS. Any newborn with a hypopigmented macule or white tuft of hair should be followed for development of the other manifestations of TS.

148.10.4 Ito's Hypomelanosis (IH)

Initially described as incontinentia pigmenti achromians, IH is a congenital multisystemic neurocutaneous disorder often characterized by neurological, musculoskeletal and eye abnormalities [49]. The disease is characterized by depigmented maculae on the limbs, where they appear as lines, and on the trunk where they appear as whorls or have a mottled appearance. These maculae appear at birth or during the first year of life. They may be asymmetric, unilateral or bilateral and may occur anywhere, except on the palms, soles and mucous membranes. No bullous or verrucous stages are seen. The skin returns to its normal colour late in childhood or early adulthood.

The most severe complications affect the central nervous system (CNS), causing mental retardation and epilepsy, which are both present in more than 50% of cases and are the two most severe and frequent disorders.

148.10.5 Piebaldism

Piebaldism is a rare autosomal dominant disorder. It is due to a defect in migration and maturation of melanoblasts from the neural crest. It is characterized by congenital, extensive and symmetrical white patches on the forehead, anterior thorax and limbs, and a tuft of white hair on the forehead. Small patches of normally pigmented skin are characteristically observed inside depigmented areas. In stable piebaldism, cutaneous depigmentation remains unchanged through life [50].

148.10.6 Albinism

Genetic abnormalities of the melanin pigment system in which the synthesis of melanin is reduced or absent are called albinism. Oculocutaneous albinism (OCA) is a group of inherited disorders of melanin biosynthesis characterized by a

generalized reduction in pigmentation of hair, skin and eyes [51]. The reduction in melanin synthesis can also be localized primarily to the eye, resulting in ocular albinism. The clinical spectrum of OCA ranges with OCA1A being the most severe type with a complete lack of melanin production throughout life. Foveal hypoplasia is invariably present and individuals with albinism often have other characteristic ocular symptoms. The degree of skin and hair hypopigmentation varies with the type of OCA but is generally reduced. The incidence of skin cancer may be increased. All four types of OCA are inherited as autosomal recessive disorders. The diagnosis is based on finding hypopigmentation of the skin and hair, in addition to ocular symptoms. Strabismus and nystagmus should be corrected and sunscreens are recommended. Regular skin checks for early detection of skin cancer should be offered. Persons with OCA have normal lifespan, development, intelligence and fertility.

148.10.7 Waardenburg Syndrom (WS)

WS is an autosomal dominant disorder classified into four types. WS type 1 is clinically characterized by dystopia canthorum, sensorineural hearing loss, congenital patches of achromic skin, white forelock, broad nasal root, synophrys, and heterochromia of irides.

148.10.8 Chediak-Higashi Syndrome (CHS)

This autosomal recessive condition is due to a gene defect localized in chromosome 1q. CHS is clinically characterized by lighter skin than parents and siblings in sunprotected skin areas, slate-gray hair, and periodic, life-threatening "accelerated phase" of systemic involvement, with fever, hepatic, splenic and lymph-node enlargement. Epstein-Barr induced malignant lymphoma is usually fatal.

148.10.9 Xeroderma Pigmentosum (XP)

XP is a rare, recessive disorder based on a deficiency in nucleotide excision repair (NER) or postreplication repair (PRR) [52]. The earliest manifestation of the disease is recognized as acute sun sensitivity. This is noted as irritability followed by prolonged erythema, edema and blistering. The severe actinic changes lead to early onset of skin cancers. Most patients do not reach adulthood, but die from metastatic cutaneous malignancies. Diagnosis can be confirmed by unscheduled DNA synthesis. XP must be distinguished from other so-called DNA repair deficiency syndromes, including Cockayne syndrome and trichothiodystrophy.

148.10.10 Neurocutaneous Melanosis (NCM)

NCM is a rare congenital phakomatosis characterized by a focal or diffuse proliferation of melanin-producing cells in both the skin and the leptomeninges [53]. This syndrome is believed to result from an error in the morphogenesis of embryonal neuroectoderm. Two-thirds of patients with NCM have giant congenital melanocytic nevi, and the remaining one-third have numerous lesions but no giant lesions. Patients may present with neurologic manifestations early in life secondary to intracranial hemorrhages, impairment of cerebrospinal fluid circulation, or malignant transformation of the melanocytes.

The prognosis of patients with symptomatic NCM is poor. In their follow-up of patients with large or multiple congenital melanocytic nevi, physicians should be aware of this condition, to aid in prompt diagnosis and treatment.

148.11 Birthmarks

148.11.1 Pigmented Birthmarks

148.11.1.1 Congenital Melanocytic Nevi (CMN)

CMN are thought to arise from disrupted migration of melanocyte precursors in the neural crest and occur in 0.2–2.1% of infants at birth. CMN have been arbitrarily divided into three size ranges, small, intermediate and giant with diameters of less than 1.5 cm (Fig. 148.13), 1.5–20 cm (Fig. 148.14) and over 20 cm, respectively. Colors range from brown to black and the commonest site is the lower back and thigh area. At birth they are often flat and pink or pale brown and most are small.

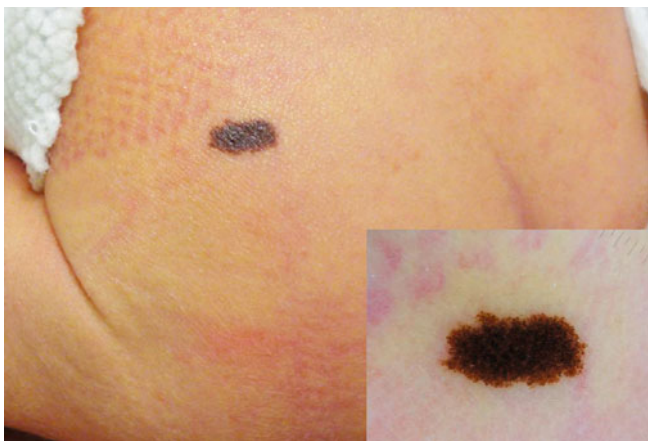

Fig. 148.13 A small (less than 1.5 cm) congenital nevus of the buttock, showing on dermoscopic evaluation an homogeneous distribution of globules (globular pattern)

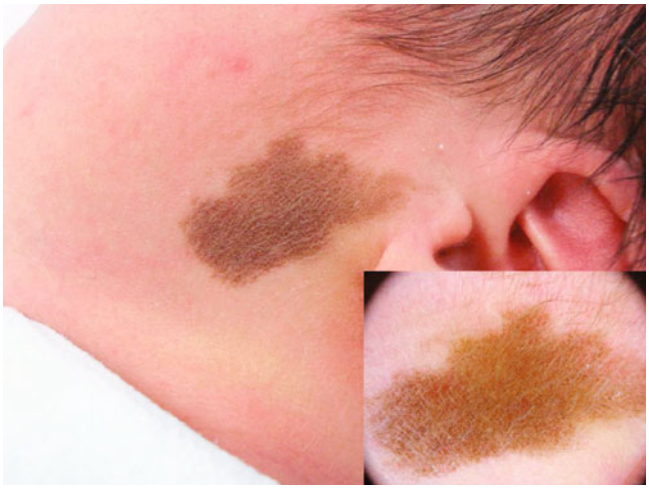

Fig. 148.14 An intermediate (1.5–20 cm) congenital nevus of the cheek, showing on dermoscopic evaluation a diffuse homogeneous pattern

Giant CMN are immediately obvious at birth. They are extremely rare and darken as the infant grows, the surface often becoming rugose or warty with nodules developing within it. The hairy component, typical of 95% of lesions, tends to become more prominent at puberty. Giant CMN of the head, neck or posterior midline area may be associated with underlying neurocutaneous melanosis. Malignant potential increases with the size of lesion and the numbers of satellite nevi near the large lesion [54]. Smaller nevi are not well studied, but malignant changes in lesions projected to grow to less than 1.5 cm in adulthood, appear to occur rarely and only after puberty. There are various treatment options for the management of giant CMN: surgical excision (total or staged), exploiting grafts, tissue expanders, dermabrasion and curettage. Curettage is best performed before 2 weeks of age [55].

148.11.1.2 Use of Dermoscopy in Management of Congenital Melanocytic Nevi

Dermoscopy is a noninvasive technique that allows visualization of structures that are not visible by clinical examination. If expertly performed, it has been shown to increase diagnostic accuracy for pigmented skin lesions, especially for malignant melanoma. Dermoscopic features of congenital nevi are equally distributed between reticular, globular (Fig. 148.13) and homogeneous patterns (Fig. 148.14).

148.11.1.3 Dermal Melanocytosis (DM)

DM includes Mongolian spots, nevi of Ota and Ito and the blue nevi.

Mongolian spots are flat, blue-gray or brown lesions with poorly defined margins, that arise when melanocytes are trapped deep in the skin. These lesions may be single or multiple and generally occur on the back or buttocks, although other areas may be involved. They may easily be mistaken for bruises. They are more common in black, native American, Asian and Hispanic populations. Most lesions fade by two years of age and do not require treatment.

Nevus of Ota, a persistent patchy blue-gray discoloration of the face, is usually unilateral and often accompanied by an ipsilateral patchy blue discoloration of sclera, conjuntiva, cornea, retina or oral mucosa. It is most common in Asians and is present at birth in more than 50% of cases of associated glaucoma. Very rarely, melanoma may occur in adult life [56].

Nevi of Ito are distinguished from those of Ota for their location in the supraclavicular, scapular or deltoid regions. It may be associated with an Ota nevus with ipsilateral or bilateral distribution.

Common blue nevi are acquired dome-shaped blue-black papules measuring less than 1 cm in diameter. They are often located on the backs of the hands or feet or nearly.

148.11.1.4 Epidermal Nevi (EN)

EN are hamartomas characterized by epidermal and adnexal hyperplasia. They may be classified into distinct variants (Table 148.18). The lesions may be deeply or slightly pigmented, have unilateral or bilateral distribution and involve any area of the skin, though they are usually located on the extremities along Blaschko lines. On the face and scalp EN

Table 148.18 Epidermal nevi

Verrucous epidermal nevus	Consists of hyperplasia of the surface epidermis and typically appear as verrucous papules that coalesce to form well-demarcated, skin colored to brown, papillomatous plaques
Sebaceous nevus	Includes many of the surface findings of verrucous epidermal nevus, but also contains malformations of the dermis, most prominently, hyperplasia and malpositioning of the sebaceous glands
Nevus comedonicus	Manifests as groups of closely set, dilated follicular openings with dark keratin plugs resembling comedones
Eccrine nevus	Is a rare cutaneous condition histologically characterized by an increase in size or number of eccrine secretory coils
Apocrine nevus	Is an extremely rare cutaneous condition found more often on upper chest and axilla histologically composed of hyperplastic mature apocrine glands
Becker's nevus	First appears as an irregular pigmentation (melanosis or hyperpigmentation) on the trunk or upper arm and gradually enlarges irregularly, becoming thickened and often hairy
White sponge nevus	Consists of bilateral white keratotic macules and plaques found especially on the buccal mucosae, but labial, lingual, vaginal and rectal mucosa may be involved

Table 148.19 Types of epidermal nevus syndromes

Well-defined syndromes, based on histopathology, extracutaneous manifestations and genetic features	– Sebaceous nevus syndrome (SNS) – Nevus comedonicus syndrome (NCS) – Becker nevus syndrome (BNS) – Phakomatosis pigmentokeratotica (PPK) – Proteus syndrome – Congenital hemidysplasia with ichthyosiform nevus and limb defects (CHILD)

are yellowish due to prominent sebaceous glands and devoid of hair. They often have a linear distribution. In infancy they are flat, tending to become warty and papillomatous in puberty in response to androgens. Most EN remain stable. However, benign or malignant tumors can arise within them.

Sebaceous nevi of Jadassohn (SNJ) are a well-known congenital hamartoma of the skin and its adnexa. They can present as isolated lesions or as rare, more complex syndrome. Sebaceous nevus syndrome (SNS) is characterized by a sebaceous nevus and extracutaneous abnormalities, usually involving organs derived from the neuroectoderm. At least six different types of epidermal naevi syndromes (ENS) have been identified (Table 148.19) [57].

148.11.2 Vascular Birthmarks

Vascular birthmarks are divided into vascular tumors and vascular malformations on the basis of their cellular features, clinical characteristics and natural history. Vascular tumors, also called hemangioma, are associated with vascular proliferation and can be infantile or congenital. Vascular malformations have an initial rapid proliferative phase followed by an involutional phase and they are developmental anomalies classified according to the type of vessel involved: capillary, vein, lymphatic vessel, artery or mixed malformations. They are present at birth, grow proportionally with the child and do not spontaneously involute [58].

148.11.2.1 Vascular Tumors

Hemangiomas (Strawberry Nevi)

Hemangiomas are the most common benign vascular tumors of infancy, occurring in 1.1–2.6 % of newborns (Fig. 148.15). Females are three times more likely to have hemangiomas than male infants, and prematurity increases the risk. At birth, the lesions may vary from bright strawberry-red coloured lesions to pale patches or telangiectasiae surrounded by a blanched halo maculae. Hemangiomas may proliferate for 18 months and then begin to involute. Approximately 50% of hemangiomas disappear by five years of age, and 90% by 10 years of age. After spontaneous disappearance, there may be residual epidermal atrophy, telangiectasia, hypopigmentation or scarring [59]. Despite their benign nature, hemangiomas can be complicated by ulceration, pain, infections, hemorrhage and scarring (Fig. 148.16).

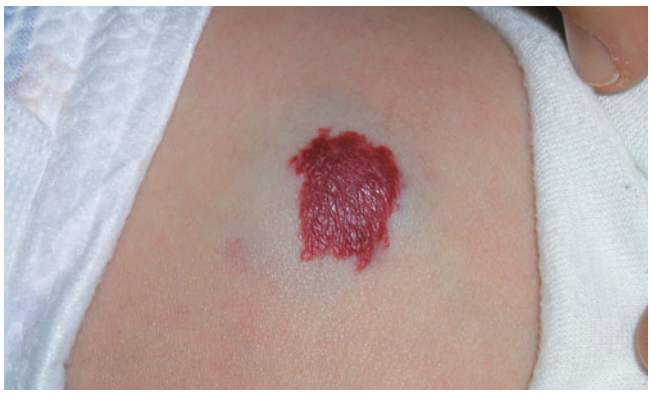

Fig. 148.15 A bright strawberry-red color hemangioma of abdomen

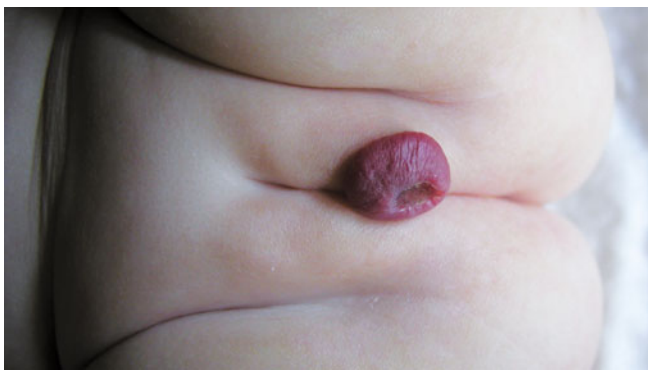

Fig. 148.16 Ulceration of a genital hemangioma

Hemangiomas that compress the eye, nose, mouth, auditory canal, or vital organs require immediate referral in the neonatal period. Uncomplicated hemangiomas should be observed with reassurance. Systemic steroids are the elective treatment for problematic proliferating hemangiomas. Propranolol may be a promising new treatment for life-threatening hemangiomas [60]. Laser treatment is also an option if the lesion threatens to interfere with the airway or the ocular axis.

Kasabach-Merritt Syndrome (KMS)

KMS is a form of consumptive coagulopathy in patients with rapidly spreading hemangiomas, vascular tumors and other malformations. It consists of thrombocytopenia, which is secondary to platelet trapping within the lesions, microangiopathic hemolytic anemia and localized consumptive coagulopathy. In some cases there is a true disseminated intravascular coagulation (DIC) [61]. KMS is not associated with classical hemangiomas, but rather kaposiform hemangioendotheliomas or

tufted hemangiomas. They are usually deep red/blue and firm and have an equal male:female distribution. If untreated, KMS can be life threatening. It requires aggressive therapy, including corticosteroids, interferon-alpha, vincristine, radiation therapy and surgery. Antiplatelet drugs and transfusion of blood products can be required too.

148.11.2.2 Vascular Malformations

Capillary Malformation (Port-Wine Stains or Nevus Flammeus)

Capillary malformation is a vascular birthmark, occurring in 0.3% of newborns, composed of mature dilated capillaries in the dermis, that appear as variably sized pink to dark red maculae. The face is most frequently affected, often unilaterally (Fig. 148.17) [62]. The lesions grow proportionally with the child and tend to darken with age. Port-wine stains in the ophthalmic distribution of the trigeminal nerve are associated with ipsilateral glaucoma.

The Sturge-Weber Syndrome

The syndrome is classically defined by the triad of glaucoma, convulsions, and port-wine stains and involves angiomas of the brain and meninges. Port-wine stains in the ophthalmic branch of the trigeminal nerve distribution (V1) are a strong indication of underlying neurological and/or ocular lesions that require on-going ophthalmologic surveillance and/or neurological or neurosurgical management [63].

Nevus Simplex

Nevus simplex is a vascular birthmark that occurs in 33% of newborns. These flat, salmon-colored lesions are caused by telangiectasias in the dermis. They occur over the eyes, scalp, and neck, with a symmetric pattern and fade when compressed.

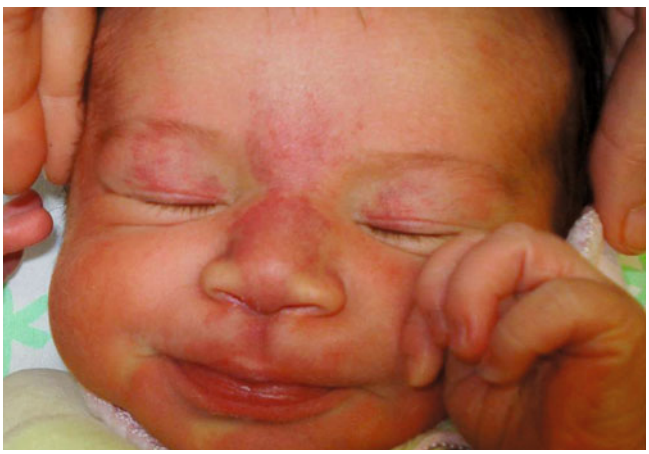

Fig. 148.17 Capillary malformation (port-wine stains or nevus flammeus) of the face in a newborn

Venous Malformations (VM)

VM are always present at birth, but they may manifest clinically later in life depending on their location. Appearances vary from a vague blue patch to a soft blue mass. When venous pressure increases, for example during crying, they usually swell, though some may remain asymptomatic throughout life. Episodic thromboses are common occur in VM, even in infants under two years of age.

Arteriovenous Malformation (AVM)

AVM is a congenital defect in which arteries and veins are tangled and not connected by capillaries. In early developmental stages, large shunts exist between future arteries and veins and a defect or arrest occurring at this stage may allow some of these connections, to persist. AVM manifests as a skin-colored mass with warm overlying skin that pulsates on auscultation. It is often asymptomatic, but symptoms depend on site. Localization in the CNS is not rare and may be associated with major neurological symptoms.

Lymphatic Malformations (LM)

LM are considered rare. There are two main types: lymphangioma and cystic hygroma. The former manifest as a group of lymphatic vessels forming a mass or lump, the latter as a large cyst or pocket of lymphatic fluid resulting from blocked lymphatic vessels. Both usually present as slowly enlarging, non-tender masses. Frequent sites are the head and neck followed by the trunk, axilla and extremities. There is a predominance of right-sided lesions. Laryngeal and oral involvement is not uncommon. In such cases and when other vital structures are involved, surgical extirpation is usually the treatment of choice [64].

148.12 Adnexal Congenital Disorders

148.12.1 Congenital Nail Disorders

The most common nail disorders in newborns and infants are listed in Table 148.20. A number of genodermatoses primarily involving the skin and mucosa are associated with more or less characteristic nail changes [65].

In epidermolysis bullosa (EB, 148.6.1.1), nail changes are seen especially in junctional and dystrophic types.

In X-linked dyskeratosis congenita, the nail plate is completely absent or hypoplastic. A pterygium formation may also be present.

Table 148.20 Nail disorders in newborns

Nail disorder	Description
Anonychia	Absence of nails; rare; may be the result of a congenital ectodermal defect or anomalies like epidermolysis bullosa, severe exfoliative diseases or infections
Micronychia	Small nail plate, shorter or narrower than normal; unusual; it is associated with syndromes or toxin exposure
Onychoschizia	Splitting of the distal nail plate into layers of the free edge. It is most noted on the big toes and thumbs, with thumb sucking being an exacerbating factor
Infantile ingrowing toenails	The thin, sharp edge of the big toenail implants into the hypertrophic lateral nail folds of the hallux. Often bilaterally, this condition improves spontaneously with age
Racket nails or brachyonychia	Nails are broad and shortened and reflect an underlying disturbance in the formation of distal phalanx
Polyonychia	Rare; several nails on the same digit due to bifurcation of the distal phalanx. A history of maternal teratogen exposure should be searched
Congenital malalignment of the great toenail	Lateral deviation of the nail plate from the longitudinal axis of the distal phalanx Spontaneous improvement occurs in approximately 50% of patients

Table 148.21 Congenital alopecia

Circumscribed alopecia	Diffuse alopecia
– Neonatal telogen effluvium – Neonatal occipital alopecia – Tinea capitis – Alopecia areata – Congenital triangular alopecia – Congenital aplasia cutis – Underlying meningocele or cystic lesion – Congenital nevi	– Alopecia areata – Marie Unna hypotrichosis – Rothmund-Thompson syndrome – Ectodermal Dysplasia – Epidermolysis bullosa

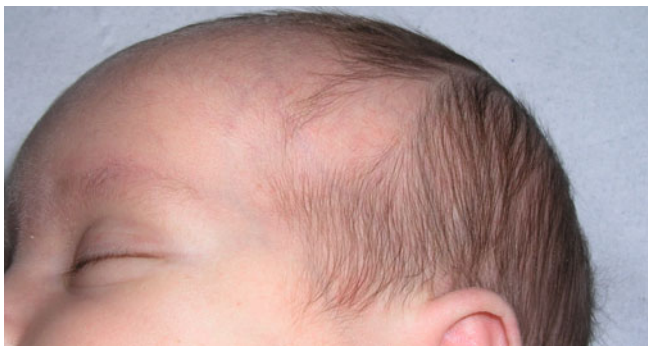

Fig. 148.18 Congenital triangular alopecia of the temporal zone

Pachyonychia congenita (PC) is a rare, autosomal dominant genodermatosis, characterized by symmetrical thickening and discoloration of the nails with a wedge-shaped, pinched-up, or claw-like appearance. Cutaneous cysts, hair abnormalities and presence of teeth at birth can be observed [66].

Nail-patella syndrome is a genodermatosis with autosomal dominant transmission associated with bone and renal disorders. The nail abnormalities recognized at birth include micronychia or anonychia, especially on the thumbs. The lunula can be triangular or V-shaped.

148.12.2 Main Causes of Alopecia in Newborns

Alopecia in newborn can be classified in circumscribed and diffuse as shown in Table 148.21. Some manifestations as neonatal occipital alopecia and telogen effluvium may be observed frequently and are considered as physiological skin changes, others are rare.

148.12.2.1 Alopecia Areata (AA)

AA is a form of non-scarring hair loss, rarely reported in the neonatal period [67].

148.12.2.2 Congenital Triangular Alopecia (CTA)

CTA is a triangular, lancet, or oval-shaped area of hair loss usually behind the fronto temporal hairline (Fig. 148.18). It may be congenital but usually appears in childhood. The hair loss may be uni- or bilateral, complete or fine vellus hairs may remain. CTA may occur as a paradominant trait.

148.12.2.3 Maria Unna Hypotrichosis (MUH)

MUH is a rare autosomal dominant pilar dysplasia, which can be seen at birth as progressive hair loss.

148.13 More Common Congenital Tumors

148.13.1 Dermoid Cyst (DC)

DC is a benign, congenital, solitary or occasionally multiple, hamartomatous tumor. It is covered in a thick dermis-like wall that contains multiple sebaceous glands and almost all skin adnexa. Hair and abundant fatty masses cover poorly to fully differentiated structures derived from the ectoderm. These growths are usually located at the midline and commonly have a deep sinus that connects to the epidermis. Common

sites include forehead, lateral eye and neck [68]. A superficial DC on the dorsal nose is referred to as a fistula and is characterized by a central tuft of hair or intracranial communication. DC can be located deep in the subcutaneous tissue, intracranially or intraorbitally. Infections, rupture and abscess formation from manipulation of DC are possible serious complications. Surgical excision is the treatment of choice in any localization.

148.13.2 Congenital Leukemia (CL)

CL associated with skin infiltration occurs in 25–30% of patients and usually presents as firm blue, red or purple nodules in a generalized distribution. CL cutis may precede other manifestations of leukemia by as much as 4 months.

148.13.3 Meningocele

Meningocele is a developmental defect related to abnormal attachment and closure of the neural tube during embryogenesis. It typically presents as a skin-coloured nodule on the scalp over the midline or along the spine. The treatment of choice is surgical excision.

148.13.4 Granuloma Gluteale Infantum (GGI)

GGI is a benign granulomatous eruption involving the gluteal region that may simulate a neoplastic process. It arises as a complication of primary irritant diaper dermatitis and typically resolves without treatment.

148.14 Other Skin Malformations

148.14.1 Congenital Aplasia Cutis (CAC)

CAC is a rare anomaly presenting with localized or widespread absence of skin at birth (Fig. 148.19). Lesions are commonly present as non-inflammatory ulcerated or membranous defects with well-defined borders of variable size, near the midline of the scalp vertex. Absent hair is a constant feature. Most single defects are oval-shaped and small (0.5–3 cm) and heal gradually during the first week after birth, leaving a residual atrophic or hypertrophic scar with alopecia [69].

For extensive scalp defects, which may extend to and involve the dura mater, differential diagnoses include: scalp infection, small meningocele, heterotopic brain or glial tissue, traumatic lesions, "en coup de sabre" morphea, sebaceous nevus, herpes simplex type 2, incontinentia pigmenti and epidermolysis bullosa. The condition may be associated with specific teratogens, intrauterine infections, epidermolysis bullosa, chromosomal abnormalities, ectodermal dysplasia, or other syndromes of malformation (Table 148.22) [70].

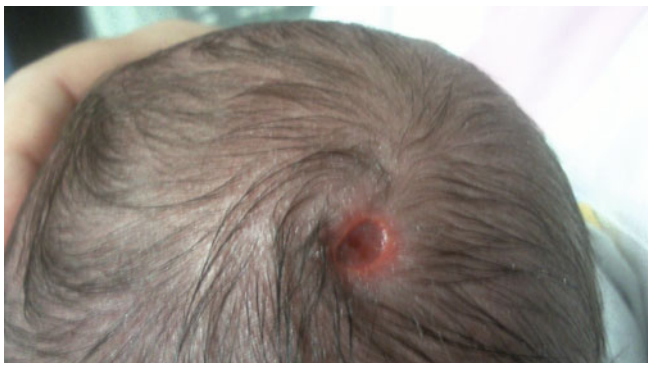

Fig. 148.19 Congenital aplasia cutis (courtesy of Dr. MC Muraca)

Table 148.22 Systemic distribution of associated findings of aplasia cutis congenita

Central nervous system	– Hydrocephaly – Meningocele – Spastic paralysis and mental retardation – Occult spinal dysraphism
Cardiovascular System	– Arterovenous malformation – Coarctation of aorta – Congenital heart defects
Gastrointestinal System	– Cleft lip- palate – Intestinal lymphangiectasia – Omphalocele
Eyes	– Myopia and cone-rod dysfunction – Oculo-ectodermal syndrome
Miscellaneous	– Ventral body wall and/or neural tube closure defects – Cutis marmorata – Piebaldism

148.14.2 Supernumerary Nipples (SN)

SN are a common congenital malformation with male predominance, consisting of extra nipples and/or related tissue besides the two nipples normally appearing on the chest. They are usually located along the embryonic milk line. Sometimes pigmented, they may be mistaken for congenital melanocytic nevi.

148.14.3 Accessory Tragus (AT)

AT is a common congenital defect linked to maldevelopment of the first and second branchial arches, which appears as a

small skin-colored tag or nodule arising near the tragus. It is composed of normal epidermis with dermal adipose tissue, pilosebaceous units, eccrine glands, elastic fibers, and cartilage.

148.14.4 Branchial Cleft Cysts (BCC)

BCC are congenital epithelial cysts, which arise on the lateral part of the neck, usually near the front edge of the sternocleidomastoid muscle, from a failure of obliteration of branchial cleft in embryonic development [71]. BCC are the most common of congenital neck masses and they are bilateral in about 2–3% of the cases. Usually asymptomatic, but, if infected, they may form a deep neck abscess or a draining fistula. Depending on the size and the anatomical extension of the mass dysphagia, dysphonia, dyspnea, and stridor, may occur.

Surgery is indicated for branchial anomalies because of lack of spontaneous regression, a high rate of recurrent infection and rare malignant degeneration.

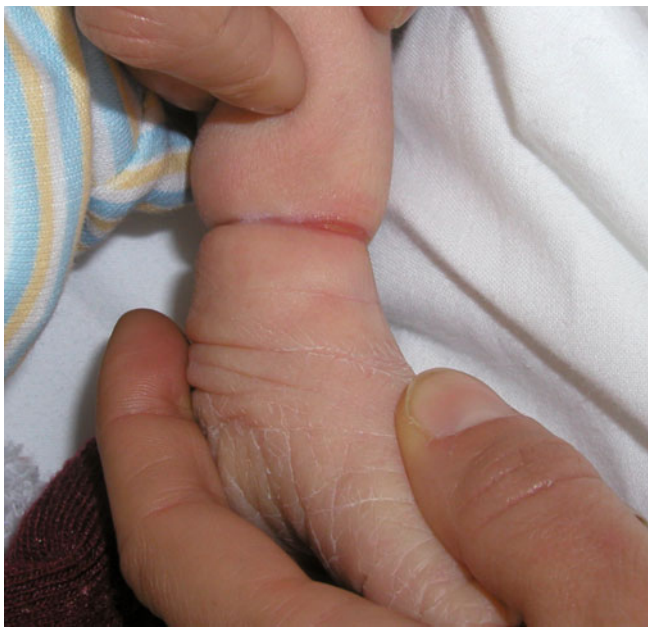

Fig. 148.20 Amniotic band syndrome (ABS) caused by entrapment of lower limb in fibrous amniotic bands during intrauterine life

148.14.5 Amniotic Band Syndrome (ABS)

ABS is a rare congenital disorder caused by entrapment of fetal parts (usually a limb or digits) in fibrous amniotic bands during intrauterine life [72]. Prognosis depends on the location and severity of the constricting bands. In mild cases, a band may result in amputations of the fingers or toes, or syndactyly (Fig. 148.20). In more severe cases, an amniotic band can become extremely constrictive leading to decreased blood supply and possible amputation of the limb. The most severe, and life-threatening complication of amniotic band syndrome is fetal death, if a band becomes wrapped around vital areas such as the head or umbilical cord.

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Appendices

Appendix A Reference intervals for laboratory tests in term and preterm newborn

Table A1 Term baby: serum electrolytes, pH, blood gases, BUN in the first hours of life

	2- to 4-hour venous blood [1]	48 ± 12 hour capillary blood [2]
pH	7.36 ± 0.04	7.395 ± 0.037
PCO_2 (mmHg)	43 ± 7	38.7 ± 5.1
PO_2 (mmHg)		45.3 ± 7.5
Htc%	57 ± 5	
Hb (g/L)	1.90 ± 0.22	20.4 ± 11.6
Na^+ (mmol/L)	137 ± 3	
K^+(mmol/L)	5.2 ± 0.5	
Cl^- (mmol/L)	111 ± 5	
iCa (mmol/L)	1.13 ± 0.08	1.21 ± 0.07
iMg (mmol/L)	0.30 ± 0.05	
Glucose (mmol/L)	3.50 ± 0.67	3.8 ± 0.8
Glucose (mg/dL)	63 ± 12	
Lactate (mmol/L)	3.9 ± 1.5	2.6 ± 0.7
BUN (mmol/L)	2.53 ± 0.71	
BUN (mg/dL)	7.1 ± 2.0	

Table A2 Preterm infant (birhtweight 1500–2500 gr). Mean ± SD venous blood electrolytes and BUN [3]

	Age 1 week	Age 3 weeks
Na^+ (mEq/L)	139.6 ± 3.2	136.3 ± 2.9
K^+(mEq/L)	5.6 ± 0.5	5.8 ± 0.6
Cl^- (mEq/L	108.2 ±3.7	108.3 ± 3.9
Ca (mg/dL)	9.2 ± 1.1	9.6 ± 0.5
P (mg/dL)	7.6 ± 1.1	7.5 ± 0.7
BUN (mg/dL)	9.3 ± 5.2	13.3 ± 7.8

Table A3 Cerebral spinal fluid

	Birthweight ≤1000 g [4]		Birthweight 1001–1500 g [4]		Term neonate [5]			
	Postnatal age (days)		Postnatal age (days)		week			
	0–7	8–28	0–7	8–28	1st	2nd	3rd	4th
	Mean ± SD		Mean ± SD					
Birthweight	822 ± 116	752 ± 112	1428 ± 107	1245 ± 162				
Gestational age at birth	26 ± 1.2	26 ± 1.5	31 ± 1.5	29 ± 1.2				
PMN%	11 ± 20	8 ± 17	4 ± 10	10 ± 19				
Glucose (mg/dL)	70 ± 17	68 ± 48	74 ± 19	59 ± 23	45.9 ± 7.5	54.3 ±17	46.8 ± 8.8	54.1 ± 16.2
Protein (mg/dL)	162 ± 37	159 ± 77	136 ± 35	137 ± 46	80.8 ± 30.8	69 ± 22.6	59.8 ± 23.4	54.1 ± 16.2
Erythrocytes/mm^3	335 ± 709	1465 ± 4062	407 ± 853	1101 ± 2643				
Leukocytes/mm^3	3 ± 3	4 ± 4	4 ± 4	7 ± 11	15.3 ± 30.3	5.4 ± 4.4	7.7 ± 12.1	4.8 ± 3.4
			Median (95 ct)					
Leukocytes/μL (term and preterm) [6]	< 28 days		2 (19)					
	28–56 days		3 (9)					

Table A4 Miscellanea of blood values

	Age	Range	Observations
ALT (IU/L) [7]	1–5 days	6–50	
Ammonia (μmol/L) [7]	1–90 days	42–144	
AST (IU/L) [7]	1–5 days	35–140	
Cholesterol (mmol/L) [8]		1.4–4.01	Term
Creatin kinase (IU/L) [9]	5–8 h 72–100 h	214–1175 87–725	Term baby
Creatinine (μmol/L)			
median (2.5–97.5 centile)	24–28 weeks GA 29–36 weeks GA	78 (35–136) 75 (27–175)	[10]

median ± 95% CI		2nd life day	7th life day	28th life day	[11]
	28	130 ± 90	84 ± 61	55 ± 40	
	30	111 ± 81	76 ± 56	50 ± 37	
	32	101 ± 74	69 ± 50	45 ± 33	
	34	91 ± 67	63 ± 46	41 ± 30	
	36	83 ± 60	57 ± 41	37 ± 27	
	38	75 ± 55	51 ± 38	34 ± 25	
	40	68 ± 50	47 ± 34	31 ± 22	

Lactate (fasting) (mmol/L) [12]	24 h 7 days	0.8–1.2 0.5–1.4	Term baby
Osmolarity (mOsm/L) in breast fed babies (mean ± SD) [13]	7 days 28 days	290.8 ± 1.78 289.7 ± 1.89	Preterm
Piruvate (μmol/L) [14]		80–150	Term baby

Table A5 Calcitonin, parathormone, calcium, magnesium and phosphate in the first hours of life in breastfed babies [15]

Hours of life	CT (pg/mL)	Pth (pg/mL)	Ca (mg/dL)	Mg (mg/dL)	Phosphate (mg/L)
1	182.2 ± 16.0	460.7 ± 48.3	9.41 ± 0.22	1.89 ± 0.06	5.45 ± 0.17
6	342.8 ± 41.4	472.0 ± 30.6	8.82 ± 0.13	2.10 ± 0.05	5.60 ± 0.18
12	476.4 ± 48.2	495.8 ± 50.3	8.50 ± 0.22	2.21 ± 0.09	5.73 ± 0.10
24	536.2 ± 55.4	633.0 ± 53.0	8.10 ± 0.19	2.42 ± 0.10	6.32 ± 0.10
72	303.6 ± 27.0	521.7 ± 25.2	9.06 ± 0.20	3.01 ± 0.12	6.83 ± 0.20
96	237.4 ± 34.5	541.2 ± 50.8	9.05 ± 0.20	2.83 ± 0.08	6.57 ± 0.10
168	175.2 ± 19.4	542.5 ± 24.0	9.30 ± 0.20	2.42 ± 0.09	6.13 ± 0.10

Table A6 Preterm babies' AST (IU/mL) and ALT (IU/mL) [16]

Corrected GA	Mean AST value (10–90 ct)	Mean ALT value (10–90 ct)
23	80 (28–1367)	7 (0–224)
25	59 (22–260)	13 (5–82)
27	37 (21–177)	12 (5–70)
33	31 (19–83)	11 (6–32)
36	40 (22–98)	13 (8–42)

Table A7 TSH, T3 and T4 values in term and preterm babies. Mean (2.5–97.5 ct) [17]

Age	TSH, mIU/L	Free T4, pmol/L	Free T3, pmol/L
7 days	3.11 (0.32–12.27)	18.0 (8.9–33.6)	6.4 (2.3–10.4)
14 days	3.01 (0.34–11.44)	17.9 (8.9–32.9)	6.4 (2.4–10.4)
21 days	2.89 (0.35–10.43)	17.8 (9.0–32.3)	6.5 (2.5–10.3)
28 days	2.80 (0.36–9.75)	17.7 (9.0–31.8)	6.5 (2.6–10.2)

Table A8 Mean (lower and upper limit) blood procalcitonin and C reactive protein [18]

	Procalcitonin (μg/L)		CRP (mg/L)	
	Term	Preterm	Term	Preterm
Birth	0.08 (0.01–0.55)	0.07 (0.01–0.56)	0.1 (0.01–0.65)	0.1 (0.01–0.64)
21–22 hours		6.5 (0.9–48.4)	1.5 (0.2–10.0)	
24 hours	2.9 (0.4–18.7)			
27–36 hours				1.7 (0.3–11.0)
56–70 hours			1.9 (0.3–13.0)	
80 hours	0.3 (0.04–1.8)			
90 hours				0.7 (0.1–4.7)
96 hours			1.4 (0.2–9.0)	
5 days		0.10 (0.01–0.8)		

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Appendix B C reactive protein and procalcitonin: Reference intervals for preterm and term newborns during the early neonatal period

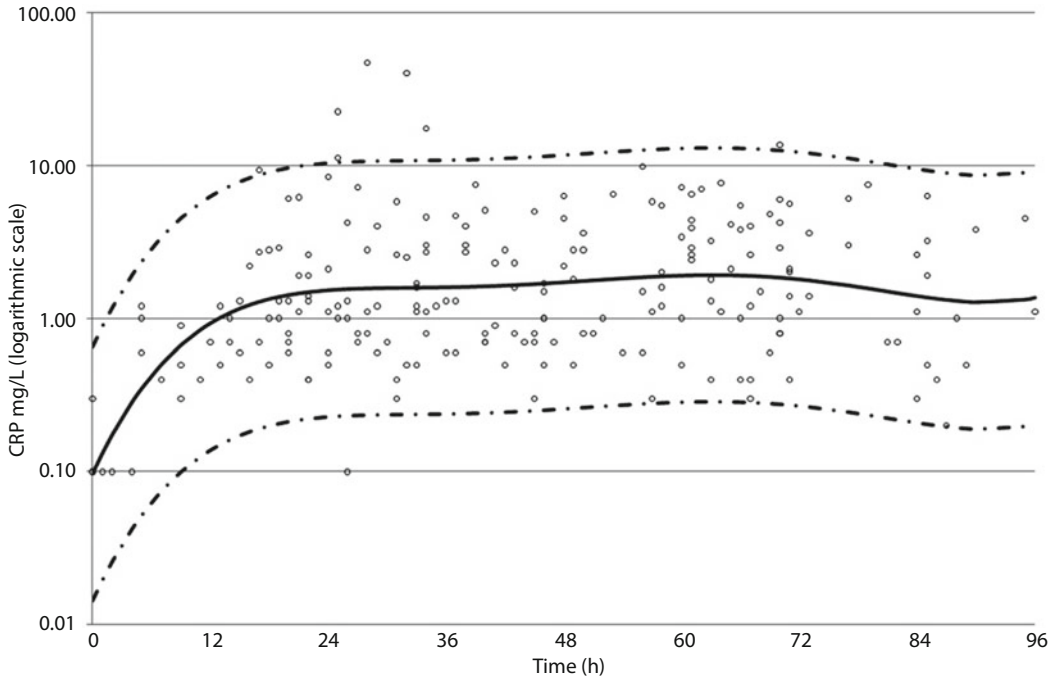

Figure B1 Age-specific 95% reference intervals for C reactive protein (CRP) in healthy term neonates from birth to 96 h of life. The circles represent single values; the dotted lines represent lower and upper limits; the bold line represents the predicted geometric mean

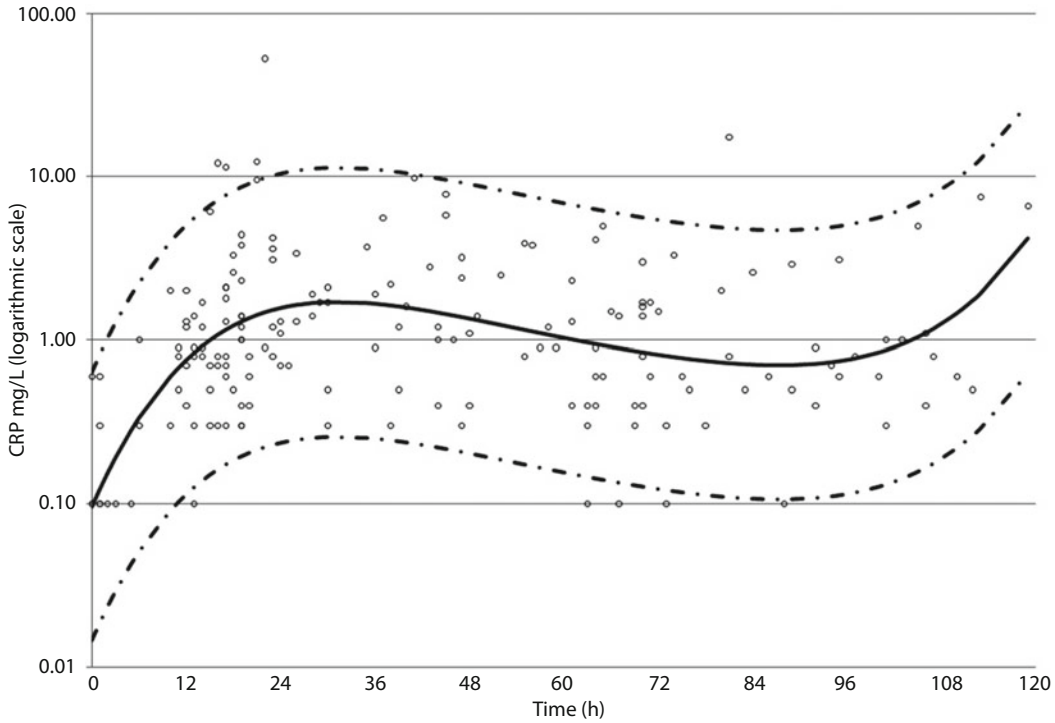

Figure B2 Age-specific 95% reference intervals for C reactive protein (CRP) in healthy preterm neonates from birth to 120 h of life. The circles represent single values; the dotted lines represent lower and upper limits; the bold line represents the predicted geometric mean

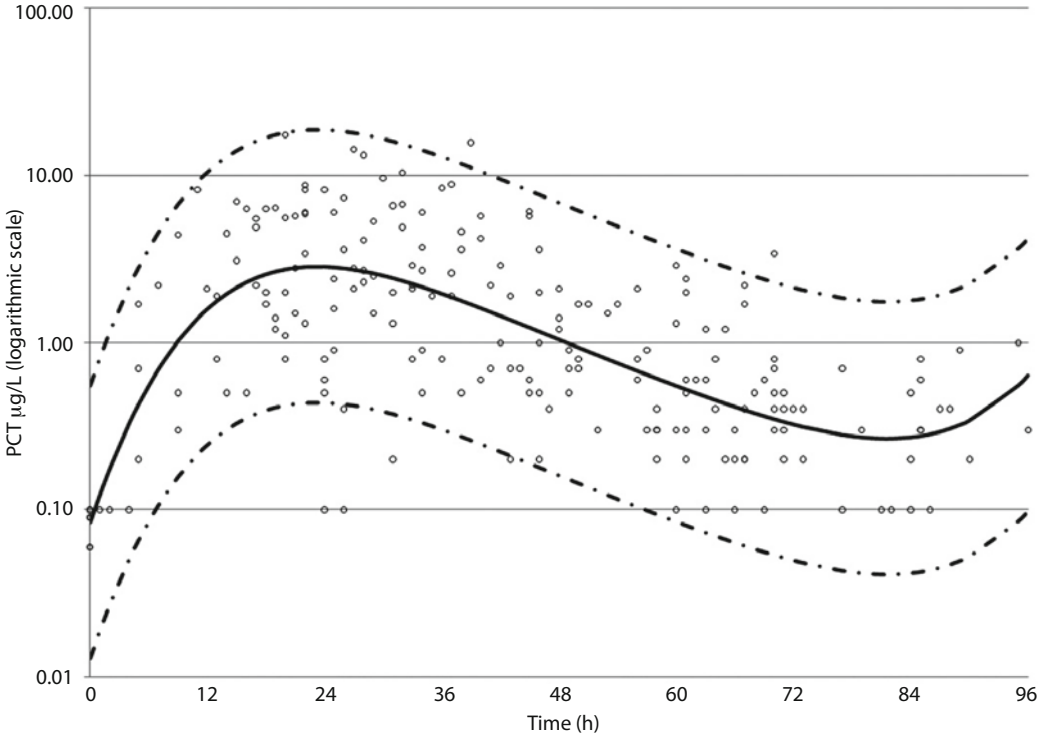

Figure B3 Age-specific 95% reference intervals for procalcitonin (PCT) in healthy term neonates from birth to 96 h of life. The circles represent single values; the dotted lines represent lower and upper limits; the bold line represents the predicted geometric mean

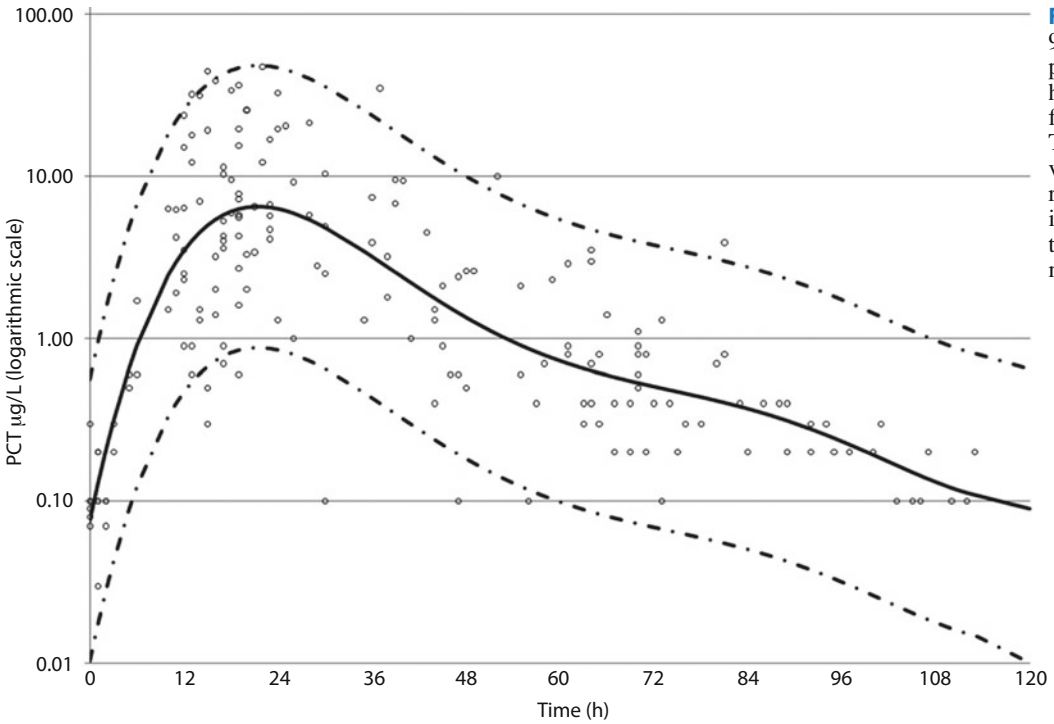

Figure B4 Age-specific 95% reference intervals for procalcitonin (PCT) in healthy preterm neonates from birth to 120 h of life. The circles represent single values; the dotted lines represent lower and upper limits; the bold line represents the predicted geometric mean

Reproduced with permission from Chiesa C, Natale F, Pascone R et al. C reactive protein and procalcitonin: Reference intervals for preterm and term newborns during the early neonatal period. *Clinica Chimica Acta* 2011;412:1053–1059.

Appendix C Distribution of neonatal birth weight, body lenght and head circumference

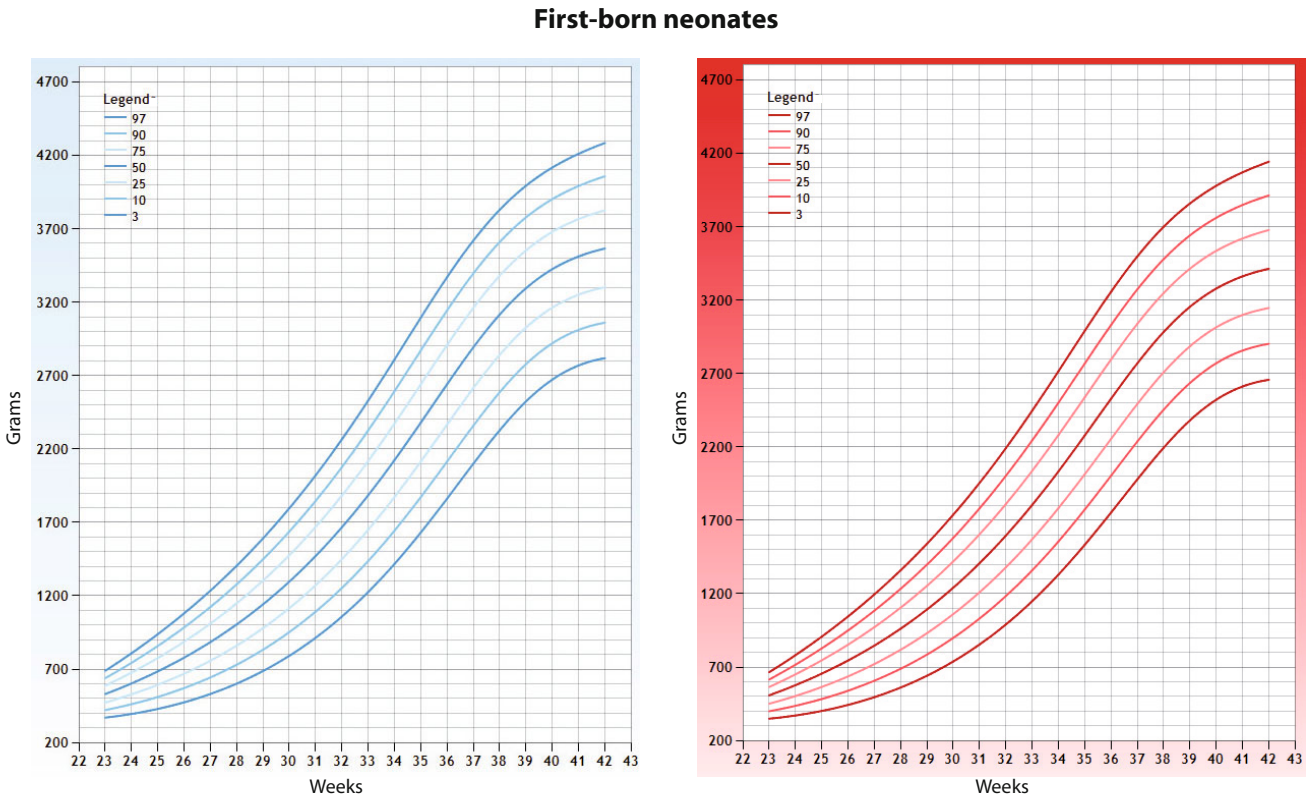

Figure C1 Distribution of birth weight in first-born neonates (*left* boys, *right* girls) for 3rd to 97th centiles (see legend)

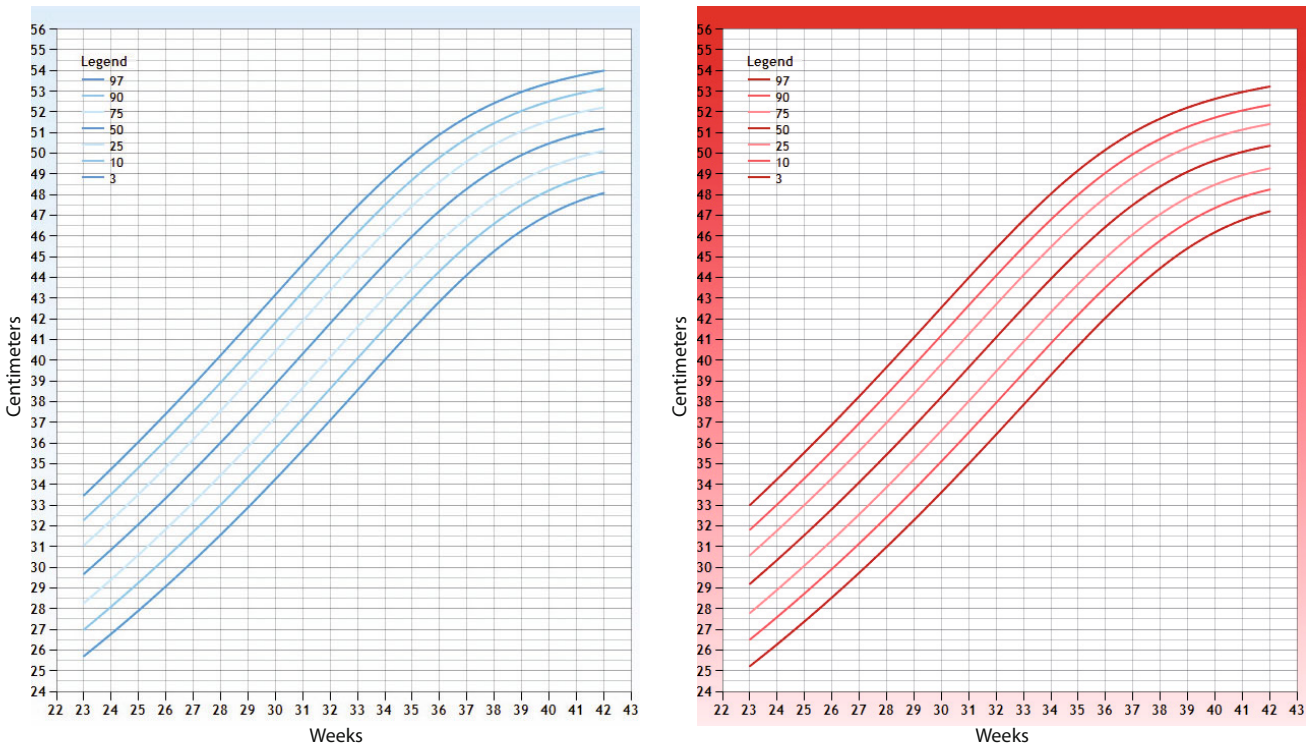

Figure C2 Distribution of body lenght in first-born neonates (*left* boys, *right* girls) for 3rd to 97th centiles (see legend)

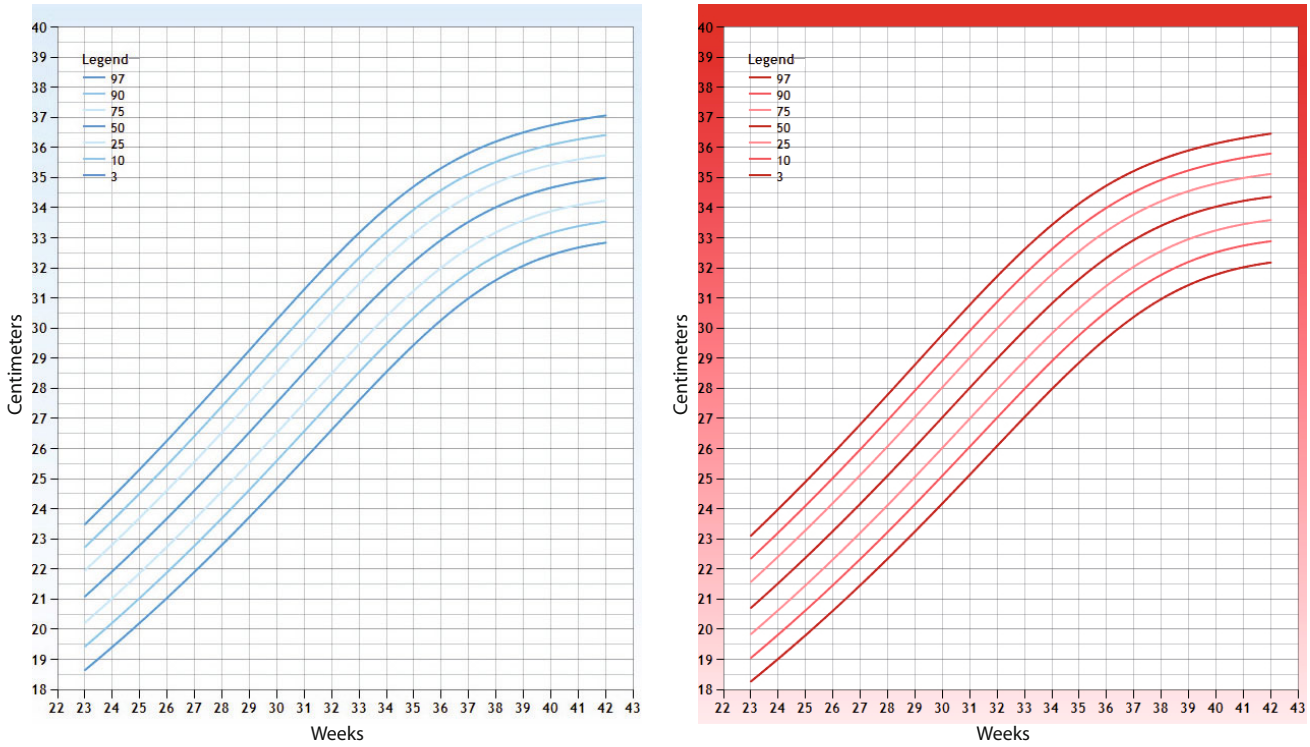

Figure C3 Distribution of head circumference in first-born neonates (*left* boys, *right* girls) for 3rd to 97th centiles (see legend)

Later-born neonates

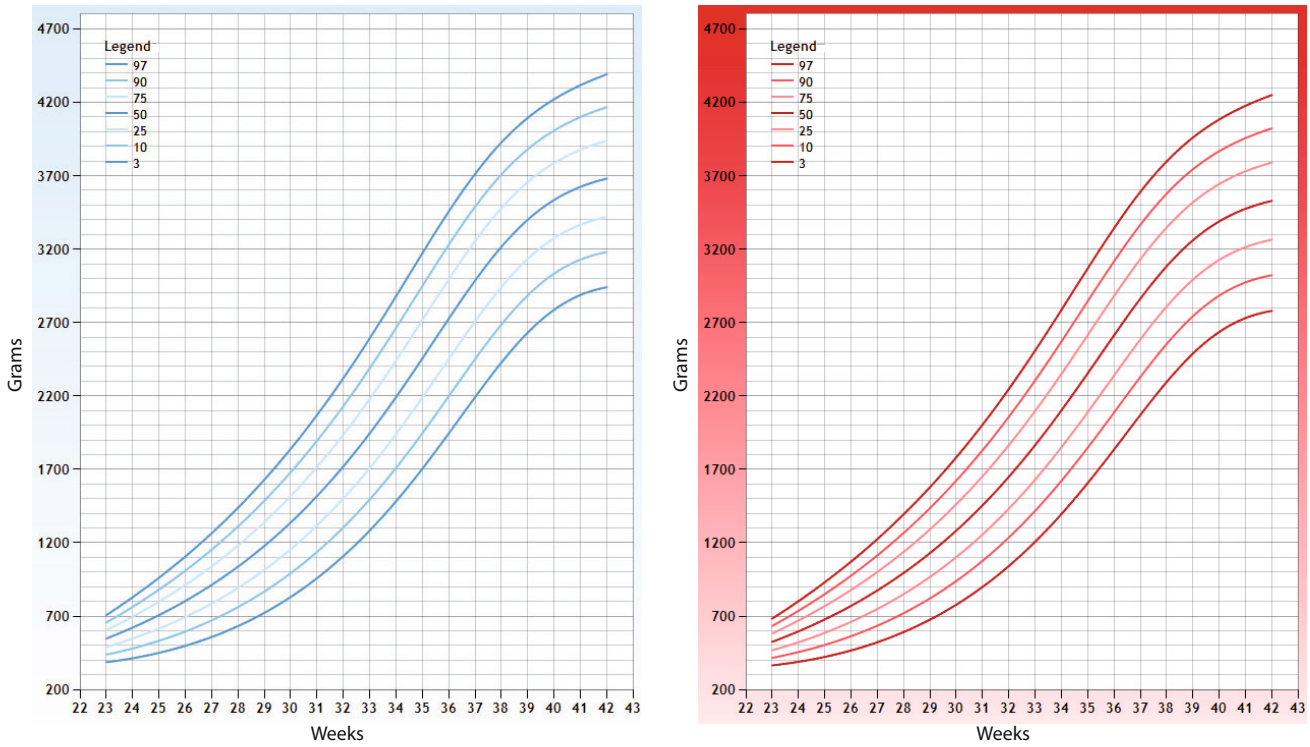

Figure C4 Distribution of birth weight in later-born neonates (*left* boys, *right* girls) for 3rd to 97th centiles (see legend)

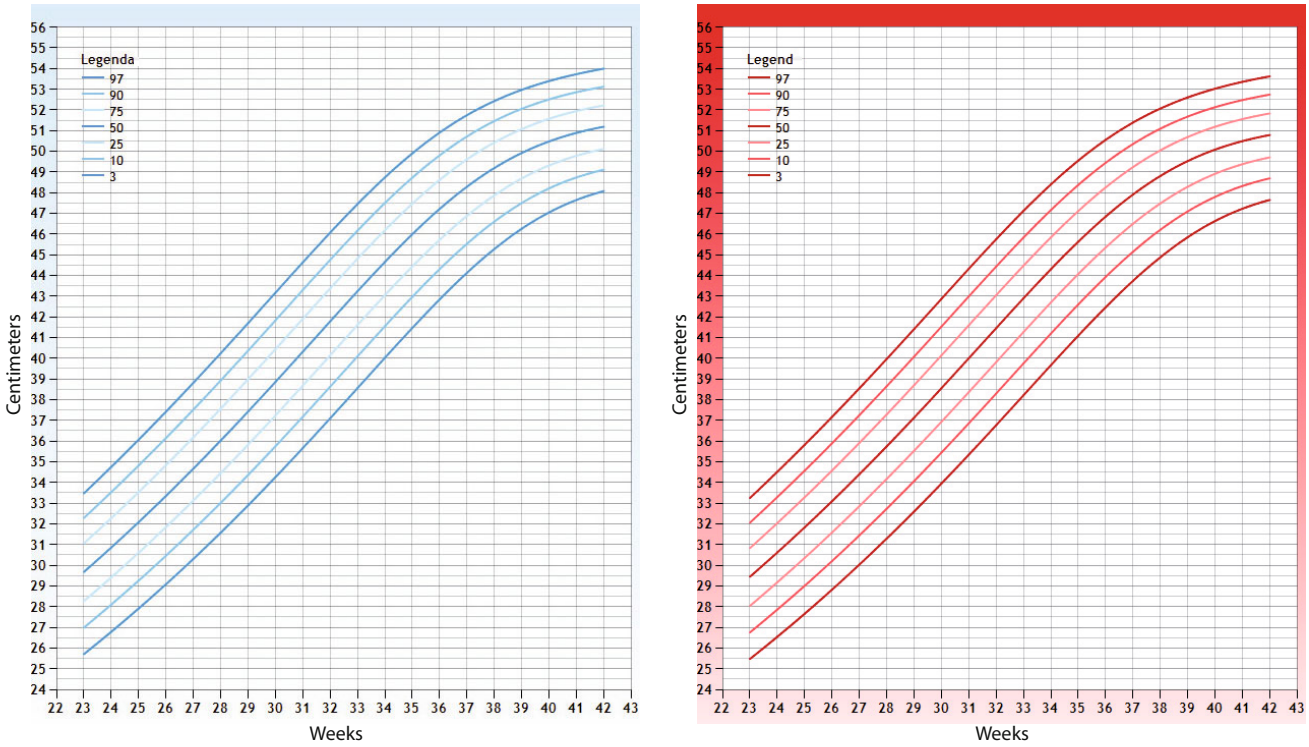

Figure C5 Distribution of body lenght in later-born neonates (*left* boys, *right* girls) for 3rd to 97th centiles (see legend)

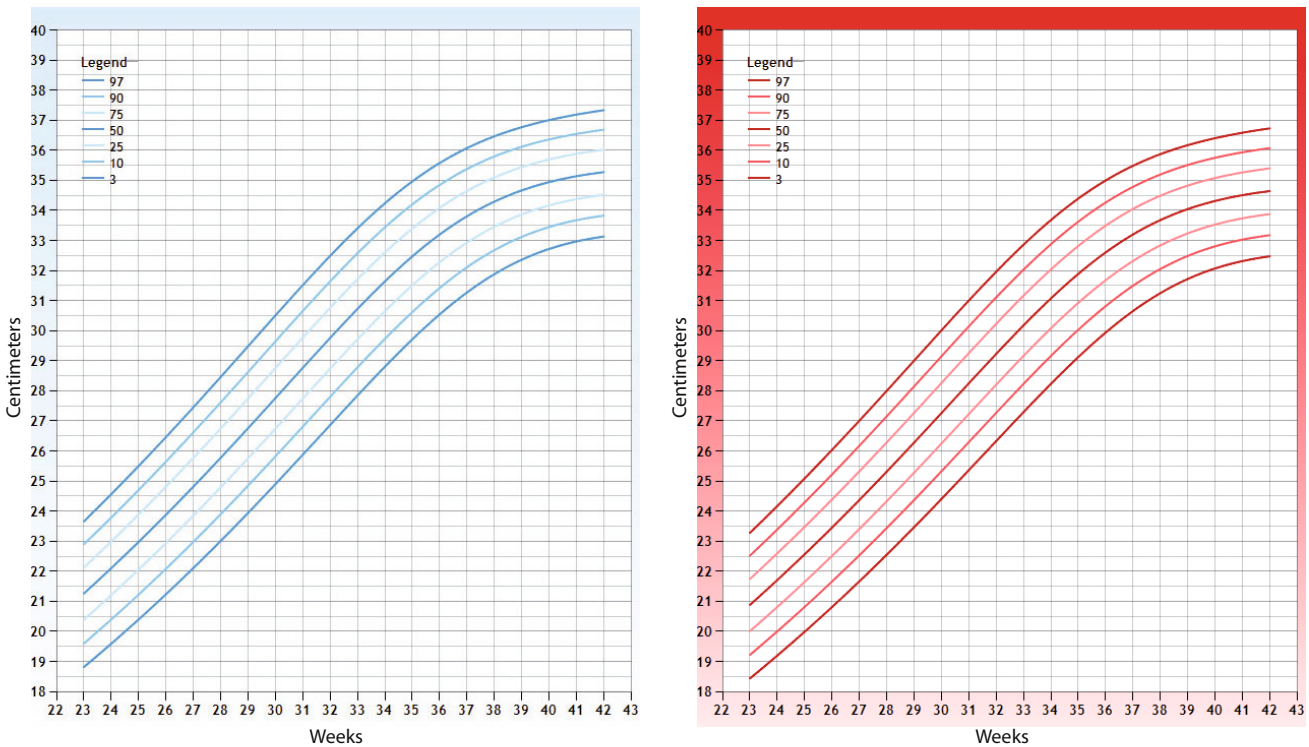

Figure C6 Distribution of head circumference in later-born neonates (*left* boys, *right* girls) for 3rd to 97th centiles (see legend)

Data of charts from Bertino E, Spada E, Occhi L et al. Neonatal anthropometric charts: the Italian neonatal study compared with other European studies. *J Pediatr Gastroenterol Nutr* 2010;51:353–361. Charts reproduced by courtesy of the Authors.

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